Laboratory Animal Allergy

Allergen exposure assessment and epidemiological study of risk factors

Albert Hollander

STELLINGEN

1. De mate van blootstelling aan luchtwegallergenen, afkomstig van proefdieren, bepaalt de kans op het ontstaan van een proefdierallergie.
   Dit proefschrift.

2. Een allergie voor huisdieren is een belangrijke indicator voor een verhoogde kans op het ontwikkelen van een proefdierallergie.
   Dit proefschrift.

3. Een aanzienlijke reductie van de allergeenblootstelling is nodig om het aantal nieuwe gevallen van proefdierallergie tot een aanvaardbaar aantal terug te brengen.

4. Een aanzienlijke reductie van de allergeenblootstelling in proefdiercentra is alleen mogelijk wanneer werkgevers, werknemers en overheid inzien dat proefdierallergie een belangrijk gezondheidsprobleem is, dat wordt veroorzaakt door het werken met proefdieren.

5. De uitspraak 'Substantial risks can no longer be expected in occupational epidemiological studies', geldt zeker niet voor studies naar beroepsallergieën.
   Wegman DH. Issues in the epidemiologic evaluation of exposure-response relationships. In: Rappaport SM, Smith TJ, eds. Exposure assessment for epidemiology and hazard control. Chelsea, MI, US: Lewis, 1991:159-174.

6. De recente organisatie van een symposium door de Nederlandse Vereniging van Arbeidshygiëne met als centraal thema de wettelijke aansprakelijkheid bij het verlenen van professioneel advies, doet vermoeden dat de commercialisering van de Arbodiensten een negatief effect heeft gehad op de kwaliteit van het door de arbodeskundigen gegeven advies.

7. Door aparte inzameling van GFT (groente, fruit en tuin) afval, stoft het overige afval veel meer dan vroeger.
   Een werknemer van een overlaadstation.

8. De capaciteit van AMV Reverse Transcriptase om bij een temperatuur van tenminste 58°C zijn activiteit te behouden, vergroot de mogelijkheden om complexe RNA moleculen met behulp van RT-PCR te analyseren.
   Dr. ir. C.C.M. van Oers, mondelinge communicatie.

9. Het idee heerst dat wij een sterke greep hebben op de natuur, maar in werkelijkheid is er sprake van een houdgreep.

10. Blank of gekleurd, arm of rijk, gehandicapt of niet, kinderen maken geen onderscheid, dat doen hun ouders voor ze.

11. Alhoewol by de Alvestêdetocht mar ien de earste wêze kin op de Bonke, binne alle reedriders dy folgje winners.
    Alhoewel bij de Elfstedentocht maar één als eerste op de Bonkevaart over de streep kan gaan, zijn alle schaatsers die volgen winnaars.

12. Het is jammer dat het nut van een goed georganiseerde en uitgeruste KNRM (Koninklijke Nederlandse Redding Maatschappij) door velen pas wordt ingezien nadat een beroep op haar diensten moest worden gedaan.

Stellingen behorende bij het proefschrift 'Laboratory Animal Allergy, allergen exposure assessment and epidemiological study of risk factors'.
Albert Hollander, Wageningen, 23 juni 1997

LABORATORY ANIMAL ALLERGY

ALLERGEN EXPOSURE ASSESSMENT AND EPIDEMIOLOGICAL STUDY OF RISK FACTORS

ALBERT HOLLANDER

Promotoren: dr. ir. B. Brunekreef
Hoogleraar in de Gezondheidsleer, meer in het bijzonder de relatie tussen milieu, arbeid en gezondheid
Landbouwuniversiteit Wageningen

P. Malmberg, M.D. PhD.
Director of the Department of Occupational Health of the National Institute for Working Life and professor linked to the Karolinska Institute, Solna, Sweden

Co-promotor: dr. ir. D.J.J. Heederik
Hoofddocent vakgroep Humane Epidemiologie en Gezondheidsleer
Landbouwuniversiteit Wageningen

ALBERT HOLLANDER

LABORATORY ANIMAL ALLERGY

ALLERGEN EXPOSURE ASSESSMENT AND EPIDEMIOLOGICAL STUDY OF RISK FACTORS

Proefschrift

ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van de Landbouwuniversiteit te Wageningen,
dr. C.M. Karssen
in het openbaar te verdedigen
op maandag 23 juni 1997
des namiddags te vier uur in de Aula.

The study presented in this thesis was supported by research grants from the Netherlands Asthma Foundation, the Ministry of Social Affairs and Employment and the European Union (contract no. BMH1-CT94-1446).

Omslag: ontwerp van Alces alces, Utrecht
Druk: Grafisch Bedrijf Ponsen & Looijen B.V., Wageningen
ISBN: 90-5485-701-3

VOOR EEF

ABSTRACT

The main objective of the study presented in this thesis was to estimate the prevalence rate of laboratory animal allergy and to determine its association with risk factors, like allergen exposure level, atopy, gender and other host factors. A cross-sectional survey was undertaken among 540 workers at 8 laboratory animal facilities. All participants completed a questionnaire and underwent skin prick testing with common and occupational allergens. Total and specific IgE measures were obtained. Prevalence rates of allergic symptoms (chest tightness (asthma), eye/nose and/or skin) due to working with rats or mice were 19% and 10%, respectively. The most common symptoms were eye/nose symptoms, 17% and 9% and asthmatic symptoms were found in 6% and 3% of the workers, respectively. The prevalence rate of sensitisation to rat or mouse allergens was 18% and 11%. Symptoms and sensitisation were strongly correlated. However, not all symptoms seemed to be IgE mediated. Rat and mouse allergy, defined as allergic symptoms accompanied by sensitisation, was highly associated with elevated total IgE ($\geq$ 100 kU/l) and positive skin prick response to common allergens. The relationship between rat and mouse allergy and positive skin prick response to common allergens, could be completely explained by a specific response to cat or dog fur allergens.

Stationary and personal air sampling was performed to identify determinants of exposure and estimate the animal allergen exposure. Animal caretakers experienced the highest exposure to aeroallergens. However, large variation within each job title was present. This may result from the wide range of tasks performed, of which handling contaminated bedding or conscious rats or mice showed the highest exposure. A relationship between allergen exposure and rat allergy became visible after excluding workers with four or more years of exposure to laboratory animals. This relationship was clearly visible for the atopic workers (having an pet allergy and/or elevated total serum IgE). The effect of exposure on rat allergy varied between atopic and non-atopic workers. Atopy should therefore be considered as an effect modifier of exposure. A higher prevalence rate of rat allergy was also found for men and smokers. However, these associations were not statistically significant.

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

BACKGROUND

The working environment often contains allergenic agents and population surveys have shown that exposure to these agents involves a high risk of developing occupational allergies[1,2]. High molecular weight agents, which are usually proteins derived from plants, micro-organisms or animals, are a major group of occupational allergens[3]. In general, the mechanism of an allergic reaction to high molecular weight agents is IgE-dependent. Clinical manifestations of an IgE-mediated occupational allergy are asthma, rhinitis, conjunctivitis, and urticaria. In the United Kingdom, a surveillance programme for occupational lung disease (SWORD) showed that asthma was the most frequently reported lung disease with an estimated proportion of 29% of the total number of occupational lung diseases[4-6]. Similar results were found in a study in the US (SENSOR)[7]. Despite being limited to cases seen by specialists in occupational and respiratory medicine, these surveillance programmes have provided crude estimates of the incidence of occupational asthma by using the number of new cases and number of workers in the various occupations. Furthermore, these surveillance programmes have provided important information on occupations at risk and suspected agents. Of all agents, urinary proteins from laboratory animals have been suggested to be the most common high molecular weight allergens causing occupational asthma[6].

Epidemiological studies among laboratory animal workers reported high prevalence rates of work related allergic asthma, rhinitis, conjunctivitis, and/or urticaria, also described as Laboratory Animal Allergy (LAA), ranging from 11 to 44 percent[8-22]. For some of the affected workers, the symptoms were so severe that direct or even indirect contact with laboratory animals became impossible[9,14]. The high prevalence rates of LAA, the severity, and the economic and personnel management consequences, resulted in an increasing research interest in LAA. Additionally, occupational asthma due to laboratory animals was recognised as a compensable occupational disease in Britain[23].

The many recently performed studies on LAA leave no question that persons working with laboratory animals are at risk of developing occupational allergy. The question of concern is what the exact magnitude of this risk is and what factors determine the risk. It seems obvious that exposure level and/or duration of exposure determine the risk of developing LAA to a large extent. This has been suggested in many reviews on occupational allergy and occupational asthma[24-28]. It has even been suggested that short periods of high exposure are possibly more important than the equivalent 'dose' accumulated at a lower exposure over a longer period of time[25,28]. However, only a few studies have suggested the presence of exposure-response relationships for

sensitisation and allergic symptoms[18,22,29]. Therefore, new studies should focus on exposure as risk factor of interest, not only because the demonstration of exposure-response relationships is a key element in establishing causality in epidemiological studies, but also because exposure can be a subject of environmental control. Recent development of immunochemical techniques for quantifying airborne allergens more accurately will undoubtedly contribute to the research on exposure-response relationships of occupational allergy.

The objective of this thesis is to study exposure-response relationships of LAA and their modification by host factors, such as atopy, gender and smoking. The majority of the cases of allergic disease among laboratory animal workers is caused by rat and mouse allergens, probably because these animals are most commonly used in experimental studies. Therefore, this thesis is focused on allergy due to working with rats or mice. Immunochemical techniques have been developed for quantifying airborne rat and mouse allergens. A major advantage in studying exposure-response relationships of occupational allergy among laboratory animal workers is that exposure to rat and mouse allergens is usually limited to the workplace.

LABORATORY ANIMAL ALLERGY

The earlier studies on LAA were all case studies[30,31], and it was not until 1974 that the first epidemiological study was performed[32]. After this study, a large number of cross-sectional studies[8-22] and a number of prospective studies have been performed[16,18,33-36]. The limitation of the majority of these epidemiological studies on LAA was that they did not distinguish between species of animals causing the symptoms, despite possible differences in exposure and potency of allergens produced by different species. In addition, the majority did not include quantitative estimates of level of exposure as risk factor in the study, but used only surrogate variables, like job title, duration of employment or frequency of contact with animals per year.

In the few studies that did distinguish between animal type, rhinitis, which was frequently accompanied by conjunctivitis, was the most commonly reported symptom related to working with rats or mice (8% - 24%). Asthmatic symptoms were the least common (3% - 10%) and were mostly found together with other symptoms. The proportion of laboratory animal workers with allergic symptoms at a given point in time, i.e. prevalence rate, given by the various studies is presented in table 1. A comparison of these prevalence rates should, however, be made with care, due to differences in definitions of rat and mouse allergy and differences in population characteristics and work environment.

Table 1. *Prevalence and incidence rates of respiratory symptoms caused by rats or mice and sensitisation to rat or mice allergens, and type of exposure characterisation in various epidemiological studies.*

			allergic symptoms						
	animal	n	eye/nose	skin	chest	total	SPT$^+$	IgE$^+$	exposure variable
Cross-sectional studies, presenting prevalence rates									
Schumacher et al.[11]	mouse	121	24% nose 12% eyes	---	4%	32%	32%	22%	no. days per month
Bland et al.[14]	rat	549	---	---	---	12%	---	---	no. hours per week, job title
Venables et al.[17]	rat	121	12%	12%	4%	18%	11%	20%	no. years employed, job title
	mouse	99	8%	6%	3%	11%	7%	40%	
Aoyama et al.[20]	rat	3518	---	---	---	25%	---	---	job title
	mouse	3246	---	---	---	26%	---	---	
Cullinan et al.[22]	rat	238	22%	15%	10%	31%	10%	---	RUA measurements
Prospective studies, presenting cumulative incidences									
Botham et al.[16]	various	249	---	---	---	14%, after one year of follow-up			none
Botham et al.[35]	various	218	---	---	---	12%, after two years of follow-up			none
Rentröm et al.[34]	various	38	---	---	---	21%, after 5 -33 months of exposure			none
Kruize et al.[37]	various	99	---	---	---	2 per 100 person years, incidence density, average 9.7 years of exposure			no. hours per week at base-line

Only a few prospective studies on LAA have been performed[16,18,33-36] and none of these studies distinguished between species of animals causing the symptoms. A Dutch retrospective study using pre-employment data[36,37] showed an incidence density of LAA of 2.0 new cases per 100 person years after an average of 9.7 years of follow-up (table 1). A small prospective studies[34] showed a cumulative incidence of LAA of 21% after 5 - 33 months of exposure. Two other prospective studies found, after recalculation of the data presented in the papers, cumulative incidences of LAA of 14% after one year of follow-up[16] and 12% after two years of follow-up[35]. The latter study showed that the majority of symptoms developed during the first year, cumulative incidence of LAA of 9%. Additionally, the Dutch retrospective study[36,37] showed a median time until development of LAA of approximately 98 months in non-atopic and 27 months in atopic workers. Interestingly, the time until development of LAA was shorter at a higher intensity of exposure.

In general, the allergic symptoms of workers with LAA occur immediately after exposure to the allergens. This immediate-type of reactivity can be assessed with Skin Prick Testing (SPT)[2,38]. Due to the fact that this immediate reaction is often IgE-mediated, in vitro tests may be used to detect specific IgE antibodies to proteins from the animals[2,38]. In several studies on LAA the presence of symptoms was highly correlated with a positive SPT response to laboratory animal allergens and/or the presence of specific IgE antibodies to these allergens[10-13,17,19,21,22]. These correlations were stronger when asthmatic symptoms were considered. The high correlations between allergic symptoms and sensitisation, suggests rat and mouse allergy to be a 'classical' example of symptomatic type I allergy. Anaphylaxis due to laboratory animals is, however, rare and has only been reported following bites by rodents or from needle injury[32,39].

Not all symptoms seemed to be IgE mediated[40], since some workers reported symptoms without detectable specific IgE or a positive SPT response. These symptoms after contact with laboratory animals were predominantly found in atopic individuals and may be the result of a non-specific hyperresponsiveness to animal-derived proteins or other agents, such as dust, disinfectants or ammonia, which are present simultaneously[21,41]. In some cases symptoms may be due to extrinsic allergic alveolitis caused by animal exposure. However, extrinsic allergic alveolitis among laboratory animal workers seems extremely rare, since only one case caused by exposure to rats has been reported so far[42].

In epidemiological studies on occupational allergy it is important to ensure that reported allergic symptoms are indeed provoked by the aeroallergen under study. In recently published guidelines for the epidemiological assessment of occupational

asthma[43], it has been suggested that occupational asthma or in general occupational allergy should not be assessed solely by the prevalence of symptoms using a questionnaire, which is a sensitive but a-specific tool[44], but should be used in combination with the results of SPT or specific IgE tests or serial Peak Expiratory Flow (PEF) measurements.

EXPOSURE TO RAT AND MOUSE AEROALLERGENS

RAT AND MOUSE ALLERGENS

The major sources of rat and mouse allergens are the excreta and secreta of the animals, which become airborne, often on dust particles, and are inhaled by those working with them. Urinary proteins are extremely allergenic[45-49] and may comprise most important allergens found in airborne dust in laboratory animal facilities[46,49]. The allergenicity of extracts of fur or epithelia of rats and mice, which are also commonly used in in vivo or in vitro tests for rat or mouse allergy, is mainly due to the presence of proteins that are also found in urine or saliva[46-49].

Electrophoresis and immunoblotting using IgE from sensitised laboratory animal workers revealed the presence of two 'major' rat urinary allergens, *Rat n* IA (20-21 kd) and *Rat n* IB (16-17 kd)[45,49-51]. Rat albumin (68 kd) also has been shown to possess some allergenicity[49,51,52], but has not been identified as 'major' rat urinary allergen. In mouse urine two 'major' mouse urinary allergens have been found, *Mus m* I (17-21 kd) and *Mus m* II (16 kd)[46,50,53-55].

MEASUREMENT METHODS

Various methods have been developed in order to measure laboratory animal allergens in the air. The first methods developed were rather insensitive, and high volume samplers had to be used in order to collect large enough amounts of allergens[56,57]. The development of more sensitive immunoassays reduced the volume of air necessary to sample and made personal sampling with flow rates at or below 2 l/min possible. In various research institutes methods have been developed to measure airborne Rat Urinary Allergens (RUA)[58-64] and Mouse Urinary Allergens (MUA)[65] in personal inhalable dust samples. However, the absolute allergen levels derived from these various methods should be compared with care[64]. The allergen levels presented in different studies may depend largely on the reference allergens, e.g. one 'major' urinary allergen[61,62,64] or a pool of urinary allergens[58-60,63,65], and on type of antibodies used, e.g. antibodies derived from sensitised workers[60,62,63], or polyclonal[58,59,65] or monoclonal antibodies[61,64] derived from immunised animals. Furthermore, differences in allergen level could result from type of assay used, e.g. inhibition[60,62,63,65],

sandwich[58,61,64] or indirect immunoassay[59], and from differences in dust sampling equipment and extraction method[66].

DETERMINANTS OF EXPOSURE

Despite the large number of methods developed to measure laboratory animal allergens in air dust samples, little is known about the determinants of exposure to these allergens. Two larger studies[63,67] showed that animal caretakers experienced the highest exposure, followed by animal technicians. However, the large variability in exposure within each job title was striking and indicated that information regarding the job performed is probably not sufficient to be used as a measure of exposure in epidemiological studies. This variability is probably due to the wide range of tasks performed[58,62,67] and the intermittent pattern of exposure. In addition, some of this variability may be explained by differences between animal rooms and between animal facilities, e.g. number of animals in the room, bedding material used and type and level of ventilation[68]. All these factors underline the complexity of exposure assessment for laboratory animals workers. Therefore, detailed information on the determinants of exposure to rat and mouse urinary aeroallergens is necessary for epidemiological studies in order to evaluate exposure-response relationships, as well as for occupational hygiene studies to evaluate the effectiveness of measures intended to reduce the allergen exposure.

EXPOSURE-RESPONSE RELATIONSHIPS

In spite of the large number of epidemiological studies on LAA, little is known about the quantitative relationship between the level of allergen exposure and risk of developing LAA. Several studies on rat and mouse allergy (table 1) have addressed this issue using various measures of exposure to laboratory animal aeroallergens. Crude estimates of exposure, like job title[14,17,20], duration of employment[17] or frequency of contact with animals per month[11], could not be related to the prevalence rate of allergic symptoms. A more detailed measure of exposure, i.e. the number of hours per day or week an individual had worked with animals, showed that allergic symptoms were more prevalent in workers working more hours with the animals[14]. However, this finding was only present in the lightly/moderately exposed workers, i.e. scientists and biotechnical personnel, and not in the heavily exposed workers, i.e. animal caretakers.

Recent development of immunochemical techniques for quantifying airborne laboratory animal allergens has provided means to study exposure-response relationships of LAA. These techniques have been applied in two studies[18,22]. In one of

these studies three exposure categories were distinguished, based on a large number of full-shift personal air measurements[22]. Allergic skin symptoms were significantly more present at medium (prevalence rate of 12%) and high exposure levels to RUA (prevalence rate of 17%) compared to the category with low exposure levels (prevalence rate of 3%). However, no relationship was observed for chest, eye, and nasal symptoms. In the other study[18], the estimated task-specific rat antigen concentration was multiplied by the duration of performed tasks. This measure of exposure was positively associated with the prevalence rate of LAA symptoms. However, in this study detailed information on the exposure levels used in the analyses was not available. Furthermore, no distinction was made between species of animals causing the symptoms, probably introducing misclassification bias. On the whole, results of both studies indicate the importance of measuring personal aeroallergen exposure in population studies on LAA.

HOST FACTORS

Not all subject develop occupational allergy given the same degree of exposure. These differences may be due to various factors related to a person, i.e. host factors, and may modify a possible exposure-response relationship. Of the host factors atopy, i.e. an inherited tendency to produce IgE antibodies to inhaled allergens, has been found to be a strong modifier of the risk of developing LAA[8,11,12,14,17,19,21,34,35,69-71]. However, a variety of criteria have been used to determine the atopic status of an individual worker, like history or family history of asthma/allergy, elevated total IgE, positive SPT response to common allergens or abnormal lung function defined as FEV_1/FVC ratio smaller than 80%. Additionally, different panels of common allergens have been used in the studies using skin prick tests. The strength of the association of atopy and LAA seems to depend on the criteria used to determine the atopic status of an individual worker[19,21,34,69,70]. For example, in a study among fifty-six laboratory animal workers[21], family history of atopy was positively associated with the presence of LAA symptoms (OR = 6.7, 95% CI 2.0 - 23). However, the association with atopy defined as a positive SPT response to common environmental allergens was much stronger (OR = 9.3, 95% CI 2.4 - 37). Interestingly, allergy to pets seems to be a strong risk factor of LAA. When the response to dog and horse was excluded from the panel of environmental allergens, the association was much weaker (OR = 3.3, 95% CI 0.7 - 16).

Little is known about other host factors being risk factors of LAA. There is conflicting evidence regarding the role of smoking in the sensitisation to occupational allergens. Some studies[22,71,72] have found sensitisation to laboratory animals allergens to be more

common in smokers, while several other studies[10,12,14,15] failed to demonstrate this relationship. The discrepancy of these results may be partly due to the cross-sectional design of most studies. It is possible that smoking habits were influenced by the development of symptoms or that subjects allergic to common allergens would less easily pick up the habit of smoking.

In previous studies on occupational allergy, the effect of gender received relatively little attention. Only two studies have previously studied the effect of gender on the prevalence rate of LAA [14,72], but no differences in prevalence rates had been found between men and women. However, general population studies have found sensitisation to common allergens to be more prevalent in men[73-77].

AIM OF THE THESIS

The aim of this thesis is to study exposure-response relationships of LAA and their modification by host factors, such as atopy, gender and smoking. Several research questions were derived from this aim.

1. To what level of airborne RUA and MUA are Dutch laboratory animal workers exposed?
2. Which factors, e.g. job title or task performed, are affecting the RUA and MUA exposure levels?
3. What is the prevalence rate of LAA in the Netherlands?
4. Is the level of animal allergen exposure related to the prevalence rate of LAA?
5. Which host factors are associated with the prevalence rate of LAA and to what extent do they influence the slope of the exposure-response relationship?

STUDY DESIGN

In order to answer these research questions, a cross-sectional study among laboratory animal workers was performed. This cross-sectional study was the 'base-line' part of a larger follow-up study. The 'base-line' survey consisted of five parts.

1. A questionnaire was administered by the participants. This questionnaire was based on a Dutch version of an internationally accepted respiratory questionnaire[78], which has been used previously in other studies on occupational asthma in the Netherlands[29,79]. In addition, questions were asked about personal history of allergic symptoms to common allergens, history of allergic symptoms to laboratory animals and hyperresponsiveness due to various environmental agents. Other questions included in the questionnaire regarded smoking history and intensity of contact with laboratory and domestic animals.

2. Skin prick testing was performed using five common allergens (house dust mites, grass pollen, tree pollen, cat fur and dog fur) and six occupational allergens (rat urine, rat fur, mouse urine, mouse fur, rabbit fur and guinea pig fur), and positive and negative controls.
3. Measurement of total IgE and specific IgE to rat and mouse urinary allergens were performed.
4. Lung function was measured by spirometry. In addition, the workers recorded their peak expiratory flow four times a day, during a period of fourteen days.
5. Exposure measurements were performed in all participating facilities. In order to estimate the allergen exposure levels, two 'sandwich' enzyme immunoassays have been developed to measure RUA and MUA. Polyclonal IgG antibodies from specific rabbit anti-RUA and anti-MUA antisera were used to capture and quantify RUA and MUA in extracts of airborne dust samples. The reaction profile of the rabbit antibodies was compared with the reaction of serum IgE antibodies of sensitised laboratory animal workers by applying Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and immunoblotting. The exposure measurements consisted of ambient air measurements, i.e. particle size and total dust sampling, and personal inhalable dust sampling during shifts as well as during the performance of specific tasks.

STUDY POPULATION

It has been estimated that there were about 4600 workers directly exposed to laboratory animals in 1990 in The Netherlands[80]. Employees from laboratory animal facilities of four universities (sites A, B, C and D), two non-university research institutes (sites E and F), one pharmaceutical company (site G) and students of a laboratory school (site H) participated in the study. All subjects working with small laboratory animals or having contact with material from these animals were invited to participate. Of approximately 750 eligible subjects 579 (77%) participated. Questionnaires were completed by 577 subjects (99.6%), 577 (99.6%) gave a blood sample and 542 (94%) were skin prick tested. A completed questionnaire as well as SPT results were available for 540 participants. Of these 540 participants, 458 (85%) worked with rats or had done so in the past and 377 (70%) worked with mice or had done so in the past. Three hundred and forty two individuals (63%) worked or used to work with mice as well as rats. The group of 540 laboratory animal workers was used in the papers presented in this thesis. This population is approximately 10% of the total population of Dutch laboratory animal workers. In addition, the workers came from different types of laboratory animal facilities and the various job title groups

were proportionally represented in this group of workers. This group of 540 laboratory animal workers may, therefore, be considered as a representative group of Dutch laboratory animal workers.

STRUCTURE OF THE THESIS

In order to estimate the allergen exposure levels, two 'sandwich' enzyme immunoassays have been developed to measure RUA and MUA. These two methods are described in **Chapter 2**. This chapter describes the specificity of the rabbit antibodies used in these assays. SDS-PAGE and immunoblotting were used to compare the reaction profile of the rabbit antibodies to the serum IgE reaction profiles of sensitised laboratory animal workers. The assay performance has also been described in this chapter.

Exposure reduction is only possible after the determinants of exposure are identified. These determinants may also be used to estimate exposure levels in epidemiological studies. An investigation of various determinants of RUA and MUA exposure is described in **Chapter 3**.

Several research groups have developed sensitive immunoassays in order to quantify laboratory animal allergen exposure. Nevertheless, absolute allergens levels derived from these immunoassays should be compared with care, due to differences in reference allergens, type of antibodies, type of immunoassay, dust sampling equipment and extraction method. In **Chapter 4** methods to measure RUA and MUA in inhalable dust samples from three European research groups are compared.

In **Chapter 5** the prevalence rates of the various allergic symptoms attributable to rat and mouse urinary allergen exposure are presented and compared with prevalence rates found in studies in other countries. In addition, the associations of reported allergic respiratory symptoms and allergen specific atopic sensitisation are described. Finally, the association between LAA and various host factors, like gender, smoking and atopy, was quantified.

Chapter 6 describes the relationship between the prevalence rate of respiratory allergy to rats and exposure to rat urinary aeroallergens, controlling for other risk factors, like atopy, gender, and smoking.

Occupational asthma can be demonstrated by recording the PEF several times a day on days away from and at work. **Chapter 7** describes the relationships between various PEF indices and the presence of allergic symptoms due to working with rats and sensitisation to rat allergens. The various PEF indices are all focused on the differences between days with and without exposure to RUA.

Finally, in **Chapter 8** the results presented in this thesis are discussed. In this

discussion the focus is on the validity of the results. Furthermore, suggestions for prevention of LAA are given and recommendations are made for further study.

CHAPTER 2

EXPOSURE OF LABORATORY ANIMAL WORKERS TO AIRBORNE RAT AND MOUSE URINARY ALLERGENS

ABSTRACT

Background: Little is known about the exposure-response relationship of laboratory animal allergy. Since laboratory animal work comprises a large number of different - often short lasting - tasks, it is of interest to assess which activities are associated with high, low or intermediate levels of allergen exposure.

Objective: To develop and evaluate highly sensitive immunoassays in order to quantify rat and mouse urinary allergens in airborne dust.

Methods: Personal air dust samples were taken during shifts and during specific tasks in 7 laboratory animal facilities. Two sandwich enzyme immunoassays were developed, using rabbit antisera against rat and mouse urinary proteins. The rabbit antibodies were analysed by SDS-PAGE and immunoblotting.

Results: The rabbit antibodies were highly specific for rat and mouse urinary proteins and reacted with all IgE-binding allergens in either urinary protein preparation. The assays for rat and mouse urine were very sensitive, with detection limits of 0.075 ng/ml. The coefficient of variation was 12.9% for both assays.

Animal caretakers experienced the highest animal allergen exposure. A large variability in exposure within jobs was found, which may be due to differences between tasks performed during the sampling day. The highest exposure levels were found during removal of contaminated bedding from the cages. However, rat and mouse allergen exposure levels during this task varied enormously between facilities, 1.1 - 158 ng eq/m^3 and 0.63 - 2000 ng eq/m^3, respectively.

Conclusions: Both sandwich immunoassays are highly specific and sensitive and are able to identify tasks of relatively short duration with high, medium and low exposure to airborne rat and mouse urinary allergens.

---

by Albert Hollander, Paula van Run, Jack Spithoven, Dick Heederik, and Gert Doekes, Clinical and Experimental Allergy (in press)

INTRODUCTION

Laboratory animal workers are at high risk of developing work-related allergic asthma, rhinitis, conjunctivitis, and/or urticaria. Cross-sectional epidemiological studies have reported prevalence rates of these allergic conditions, also described as laboratory animal allergy (LAA), ranging from 11 to 44 percent[8,10-18,20,22]. However, little is known about the quantitative relationship between the level of allergen exposure and risk of developing LAA. Several studies[11-14,17,18,22] have addressed this question using in most cases rough estimates of exposure, like type of job or years of exposure. Only recently specific immunoassays have been developed with sufficient sensitivity to quantify laboratory animal allergens in personal samples of airborne dust[58-63]. The two studies[18,22] applying these immunoassays both found an association between level of exposure and prevalence rate of LAA, which might indicate the importance of measuring actual personal aeroallergen exposure in such population studies.

In the Netherlands a large follow-up study amongst approximately 600 laboratory animal workers is currently in progress. The aims are to study exposure-response relationships for LAA and their modification by atopy, gender, and other factors. In order to estimate the allergen exposure levels, two 'sandwich' enzyme immunoassays (EIA) have been developed to measure rat (RUA) and mouse urinary aeroallergens (MUA). Polyclonal IgG antibodies from specific rabbit anti-RUA and anti-MUA antisera were used to capture and quantify RUA and MUA in extracts of personal airborne dust samples.

Firstly, this paper describes the specificity of the rabbit antibodies by applying SDS-PAGE and immunoblotting. The reaction profile of the rabbit antibodies was compared with the reaction of serum IgE antibodies of laboratory animal workers. Secondly, the assay performance was described by testing the specificity, reproducibility and sensitivity of both assays. Thirdly, personal air samples were taken in different laboratory animal facilities during full-shift periods as well as during various short lasting tasks in order to evaluate if these assays could be used to identify job titles and/or tasks with high levels of exposure to laboratory animal aeroallergens.

MATERIALS AND METHODS

SITES

Seven laboratory animal facilities, which were affiliated to a university (sites A, B, C and D), research institute (sites E and F) or pharmaceutical company (site G), took part in this survey. Among laboratory animal workers five broad job titles and nine major tasks could be distinguished (table 1).

Table 1. Job titles and task categories in laboratory animal workers

JOB TITLES	
animal caretaker	performs the 'general care of animals' and cleans the cages and rooms
animal technician	performs experimental procedures on animals on a full-time basis
scientist	performs procedures on animals, their tissues or body fluids, but on a part-time basis
scientific assistant	assists scientists in their work and is also part-time exposed to laboratory animal allergens
supervisor	has no direct contact with animals, their tissues or their body fluids
TASK CATEGORIES	
cleaning rooms	cleaning rooms and sweeping zones (mean sampling time 58 minutes, range 10 - 128)
change into new cages	changing animals from dirty into clean cages & cleaning racks (mean sampling time 73 minutes, range 10 - 270)
cleaning out cages	removing woodchips from dirty cages (mean sampling time 56 minutes, range 7 - 218)
cage washing	bottle and cages washing in cage washing area (mean sampling time 64 minutes)
feeding	changing animal feed and water bottles (mean sampling time 57 minutes, range 5 - 186)
handling animals	handling of animals in animal room, for example tumour control, weighing, taking temperature (mean sampling time 85 minutes, range 10 - 341)
biotechnical work	immunisation, injection, blood sampling (mean sampling time 92 minutes, range 15 - 325)
experiments 1	experimental work on anaesthetised animals (mean sampling time 163 minutes, range 25 - 317)
experiments 2	experimental work on conscious animals; handling/observing (mean sampling time 161 minutes, range 85 - 267)

AIR SAMPLING AND ELUTION OF FILTERS

Inhalable dust was sampled using IOM sampling heads (IOM, Edinburgh, Scotland) with a flow rate of 2 l/min. Each sampling head contained a polytetrafluoroethylene (Teflon) filter (Millipore; pore size 1.0 μm, ∅ 2.5 cm). A total of 287 personal samples were collected during full-shift periods of 4-8 hours with an average sampling time of 6 hours. Two hundred and seventy-eight personal samples were collected during various tasks as described in table 1. Forty-six full-shift samples, where work entailed only one task, were included in these 278 samples. The mean and range of the sampling time of each task is described in table 1. No task sampling was performed at site F. All dust sampling was carried out between September 1992 and November 1993.

RUA and MUA were recovered from the filters by extraction at room temperature with 2 ml 0.15 M phosphate buffered saline (PBS, pH 7.4). Filters were successively vortexed for 2 minutes, sonicated for 2 minutes, vortexed for 5 minutes and sonicated for 2 minutes. The extract was centrifuged at 5,000 g for 15 minutes. The supernatant was collected and stored at -20°C[81].

ALLERGEN PREPARATION

Urine from Wistar rats and Balb/c mice was collected using metabolic cages. Male and female (3:1) as well as pubertal and adult animals (1:1) were used to control for sex and puberty effects on protein content and composition of urine[49]. Proteins in the pooled rat and pooled mouse urine were isolated by ALK Benelux (Houten, The Netherlands) through extensive dialysis against PBS and twice distilled water, and concentrated by lyophilisation. The protein content of the rat and mouse urinary preparations were 0.50 and 0.72 mg per mg dry weight (BCA, Pierce, Rockford, IL USA[82]). The two urinary allergen preparations were used for rabbit immunisation, sandwich EIA and SDS-PAGE. These allergen preparations were also used for skin prick testing to assess sensitisation among laboratory animal workers[83].

ANTIBODIES

Two New Zealand white male rabbits were purchased from the Broekman Institute (Someren, The Netherlands). One ml of either RUA or MUA solution (0.5 mg protein/ml) was mixed with an equal volume of Freund's complete adjuvant and injected subcutaneously into the rabbits. Booster injections were administered at 4 week intervals, with the same allergen concentrations in Freund's incomplete adjuvant. The immunoglobulin (Ig) fraction was isolated from a serum pool of each animal by ammonium sulphate precipitation, redissolved in PBS and stored at -20°C. This Ig fraction was used as capture antibody in the sandwich EIA. Part of the antibodies was biotinylated to be used as detector antibody. Biotinylation was performed by incubating 1 ml (1 mg/ml) of the antibodies with 10 µl 1 M sodium carbonate buffer (pH 8.6) and 180 µl biotin solution for 4 hours at 20°C. One mg biotin-N-hydroxysuccinimide ester (Boehringer Mannheim GmbH, Mannheim, Germany; 1008.960) per ml dimethyl sulfoxide (Sigma, St. Louis, MO USA; D-8779) was used. The biotinylated antibodies were dialysed at 4°C against 6 changes of PBS for 48 hours and stored at 4°C.

ELECTROPHORESIS AND IMMUNOBLOTTING

Lyophilised RUA (32 µg per cm gel) and MUA preparations (4 µg per cm gel) were

electrophoretically separated on a 12% SDS polyacrylamide gel system (Mini-Protean II slab cell unit, Biorad, Richmond, CA, USA) according to Laemmli[84]. Low molecular weight markers (Biorad; 161-0304) were used as standards. The lane with the markers, together with a small part of the gel containing the urine sample, was cut from the gel and stained with Coomassie Blue R 250 (Pharmacia, Uppsala, Sweden 1840-101). The other part of the gel was blotted onto nitro-cellulose (Sleicher & Schuell, Dassel, Germany; 0.22 µm pore size). After blocking of unoccupied binding with PBTG (PBS, containing 0.05% Tween-20 and 0.2% gelatine), the nitro-cellulose sheet was divided into strips of approximately 2 mm.

The strips were incubated at room temperature overnight with 140 µl undiluted serum samples from rat or mouse sensitised workers, 56 and 47 subjects with specific IgE ≥ 0.7 kU/l[83], respectively, or 1:50 diluted biotinylated rabbit anti-RUA or MUA antibodies. Bound human IgE was detected by subsequent incubations with 140 µl 1:500 diluted mouse-anti-human IgE (CLB, Amsterdam, The Netherlands), 140 µl 1:500 diluted biotinylated rabbit anti-mouse Ig, and 140 µl 1:200 diluted avidin peroxidase conjugate (both Dakopatts, Copenhagen, Denmark). Incubations were performed with PBTG as the diluent at room temperature for 1 hour and between the incubations the strips were washed with PBT (PBS, containing 0.05% Tween-20). In immunoblotting with RUA the biotinylated rabbit anti-mouse Igs were diluted in PBTG containing 5% rat serum to prevent their binding to one of the rat urine proteins. Bound rabbit anti-RUA or MUA antibodies were detected by incubations with 140 µl 1:200 diluted avidin peroxidase conjugate (Dakopatts) at room temperature for 1 hour. Peroxidase binding was in both procedures detected by incubation with 140 µl 3,3',5,5'-tetramethylbenzidine (TMB, Promega Corporation, Madison, WI, USA) for 30 minutes.

SANDWICH EIA

Polystyrene high capacity microtiter plates (Greiner, Frickenhausen, Germany; 655061) were coated with 200 µl rabbit anti-RUA or MUA antibodies (1.3 and 1.6 µg/ml PBS, respectively) at 4°C overnight. The next morning the plates were washed and incubated with PBTG at 37°C for 30 minutes. PBTG was also used as the diluent in further incubations and between the incubations the plates were washed with PBT. 200 µl of samples and 12 dilutions (in duplicate) of the standard were added to the wells. Solutions of RUA and MUA were used as standard, ranging from 0.050 to 2 ng protein/ml. The plates were incubated at 37°C for 1 hour. After washing, 200 µl 1:500 diluted biotinylated rabbit anti-RUA or MUA antibodies were added to each well, followed by incubation with 200 µl avidin peroxidase conjugate (Dakopatts) diluted

1:2,000 (both at 37°C for 1 hour), and finally an incubation with 200 µl O-phenylenediamine (2 mg/ml, containing 0.015% H_2O_2) in 0.05 M citrate/phosphate buffer (pH 4.5) at 20°C for 30 minutes in the dark. The reaction was stopped by adding 50 µl 2 M HCl. The absorbance of each well was measured at 492 nm with an EIA-reader (Thermomax microplate reader, Molecular Devices Corporation; Menlo Park, CA USA). A standard curve of the optical density at 492 nm (OD_{492}) against the log concentration of standard allergen was calculated with 4-parameter curve fitting, using the SOFTmax software package (Molecular Devices Corporation). Concentrations in test samples were expressed in ng rat or mouse urinary protein *equivalent* (ng eq) per ml in which 1 ng eq was defined as the amount of allergenic activity giving the same OD_{492} as 1 ng protein of the standard.
Animal feed (RMH-B, Hope Farms, Woerden, The Netherlands) and urine from mice or rats are potential sources of cross-reacting allergens in the rat and the mouse assay. Both assays were tested for specificity using allergen preparations of these sources.
Instead of using the lowest measurable standard as detection limit of the assays, the detection limit was estimated by analysing extracts of 126 blank filters. These filters underwent the same procedures as the loaded filters except for the actual air sampling. The average allergen concentrations of these extracts, using extrapolation of the 4-parameter curve, was 0.026 ng eq/ml (SD = 0.028) for the RUA assay and 0.018 ng eq/ml (SD = 0.023) for the MUA assay. Only extracts of air samples with a concentration higher than the mean concentration of these blanks + 2 SD were considered positive, which implied a detection limit of 0.075 ng eq/ml for both assays.

STATISTICAL ANALYSIS

The mean and standard deviation of the RUA and MUA exposure were calculated based on log-normally distributed concentrations, giving the geometric mean (GM) and geometric standard deviation (GSD). A value of two-thirds of the detection limit (0.17 mg/m^3 for inhalable dust, 0.23 ng eq/m^3 for RUA and MUA levels of full-shift samples, 0.94 ng eq/m^3 for RUA and MUA levels of task samples) was assigned to samples with undetectable dust or aeroallergen concentrations. The different detection limits for the shift and task samples were due to differences in sampling time.

RESULTS

ELECTROPHORESIS AND IMMUNOBLOTTING

The specificity of the anti-RUA and MUA rabbit antibodies was examined by SDS-PAGE and immunoblotting. The reaction profile of the antibodies was compared with that of serum IgE antibodies of laboratory animal workers, as shown in figure 1 and 2

for 25 representative sera. Coomassie staining of rat urine after SDS-PAGE revealed two strongly staining bands at 66 and 15 kd and seven weaker bands (figure 1). For mouse urine two strongly staining bands at approximately 22 and 18 kd and one weakly staining band with molecular weight around 14 kd were visible (figure 2).
In the immunoblots the number of reactive proteins that could be distinguished increased with increasing IgE titre of the test serum. Sera with high titres (≥ 17.5 kU/l) reacted with all nine rat urine proteins and with four mouse urine proteins. For rat urine the strongest reactions were observed with the 15, 24 and 66 kd proteins, and the most pronounced reactions to mouse urine appeared to be directed to proteins of 18, 22 kd and to a component of 40 kd. This 40 kd component was not detected by staining with Coomassie Blue. The anti-RUA (figure 1, track R_a) and anti-MUA rabbit antibodies (figure 2, track M_a) reacted with all IgE-binding components in rat and mouse urine, respectively. In the test for cross-reactivity, the anti-MUA antibodies (figure 1, track M_a) also showed a weak reaction to the 15 kd and 66 kd rat urine proteins, whereas the anti-RUA antibodies (figure 2, track R_a) showed no reaction with any of the mouse urine allergens.

ASSAY PERFORMANCE

As shown in figure 3, steep dose-response curves were obtained for both assays using the standard preparations at low concentrations of protein. Maximum OD_{492} values (2.0 - 2.5) were reached at approximately 1.5 ng/ml and the OD_{492} values obtained with the lowest standard concentration (0.05 ng/ml) were consistently elevated compared to that of the reagent blank (no rat or mouse urine). The high specificity of the anti-RUA and anti-MUA antibodies was also confirmed in the sandwich EIA (figure 3). The MUA assay showed to be extremely specific, and was 25,000-fold less sensitive for RUA than for MUA (figure 3 bottom). The RUA assay was even more specific, and was 325,000-fold less sensitive for MUA than RUA (figure 3 top). Animal feed extracts showed in both assays only minor reactions (figure 3).
Extracts of airborne dust samples were tested at multiple dilutions. The dose-response curves of these samples were essentially parallel to the standard curves, and thus similar concentrations were calculated from the OD values found at different dilutions (data not shown). The reproducibility of the assays was estimated by computing coefficients of variation (CV) for duplicate analysis of RUA and MUA in extracts of personal air samples. One hundred and forty eight and one hundred and eighty nine air samples with detectable concentrations of RUA and MUA, respectively, were analysed in duplicate on two different days. The CV of the analysis, independent of allergen level, was 12.9% for both assays.

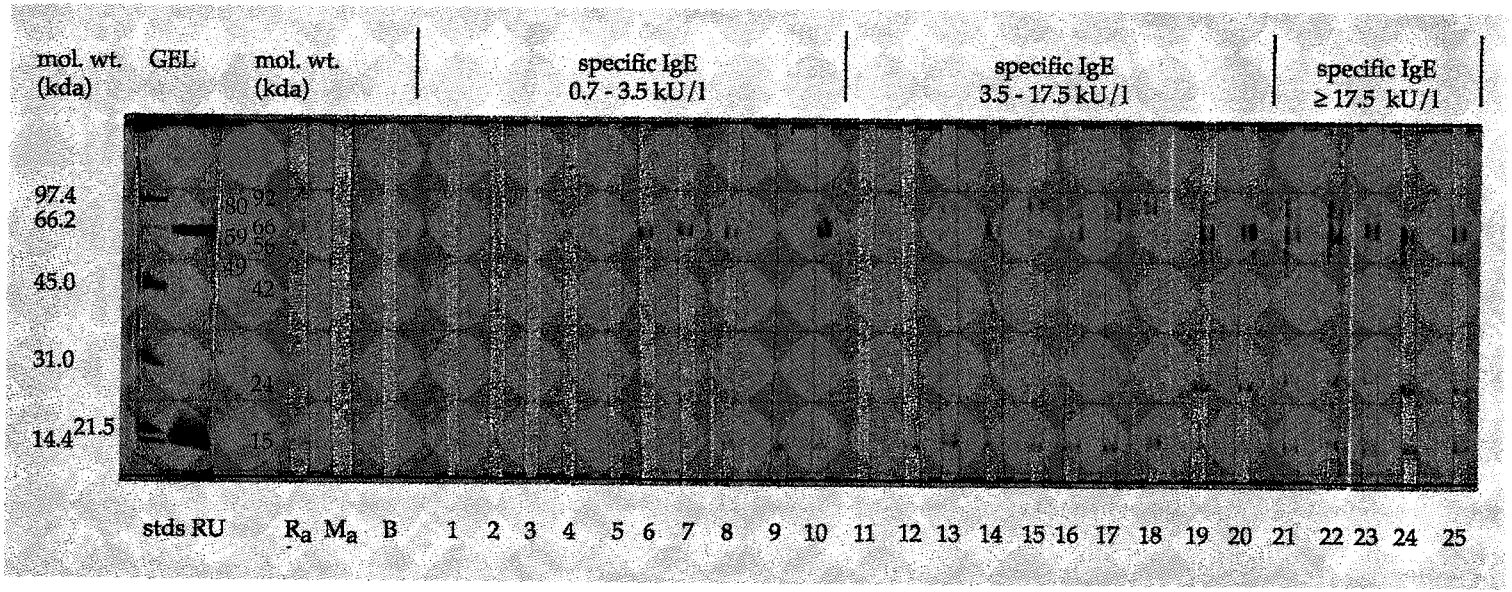


Figure 1. *Analysis of rat urine proteins by SDS-PAGE (12% resolving gel, reducing conditions) and immunoblotting. Twenty five representative sera (track 1 through 25), divided into three groups with increasing IgE titre. Mol. w., Molecular weight; kda, kilodaltons; stds, standards; RU, rat urine; R_a, rat urine antiserum; M_a, mouse urine antiserum; B, negative control.*

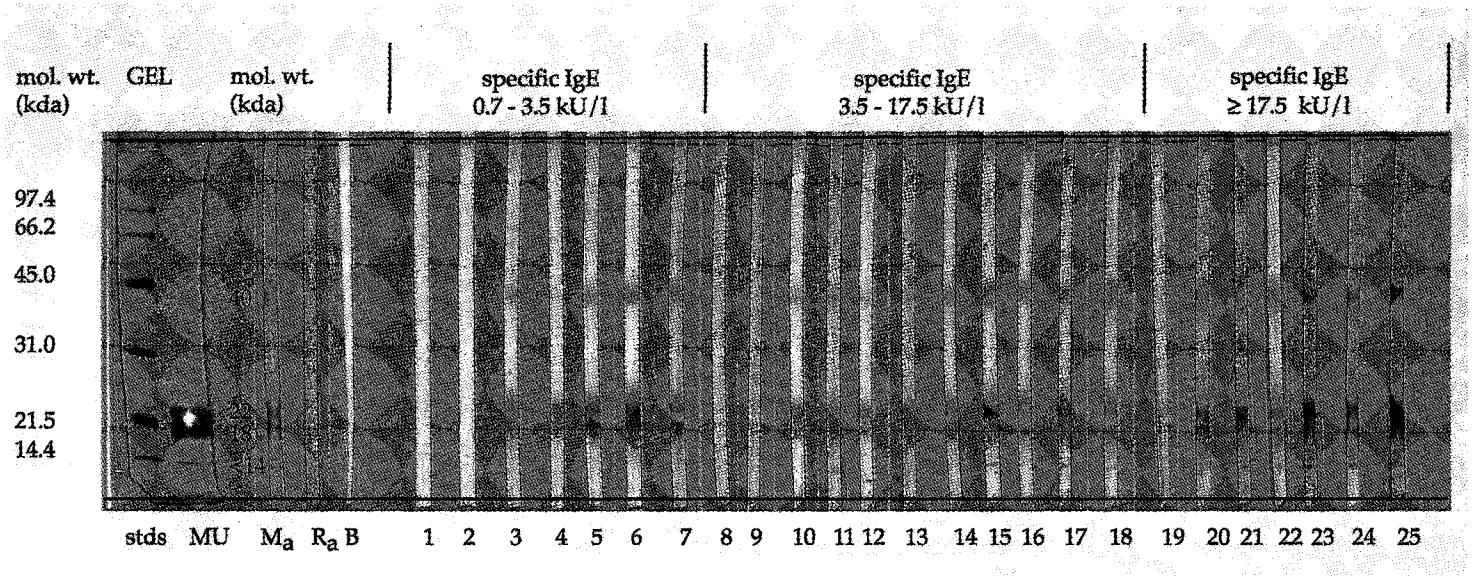


Figure 2. *Analysis of mouse urine proteins by SDS-PAGE (12% resolving gel, reducing conditions) and immunoblotting. Twenty five representative sera (track 1 through 25), divided into three groups with increasing IgE titre. Mol. w., Molecular weight; kda, kilodaltons; stds, standards; MU, mouse urine; M_a, mouse urine antiserum; R_a, rat urine antiserum; B, negative control.*

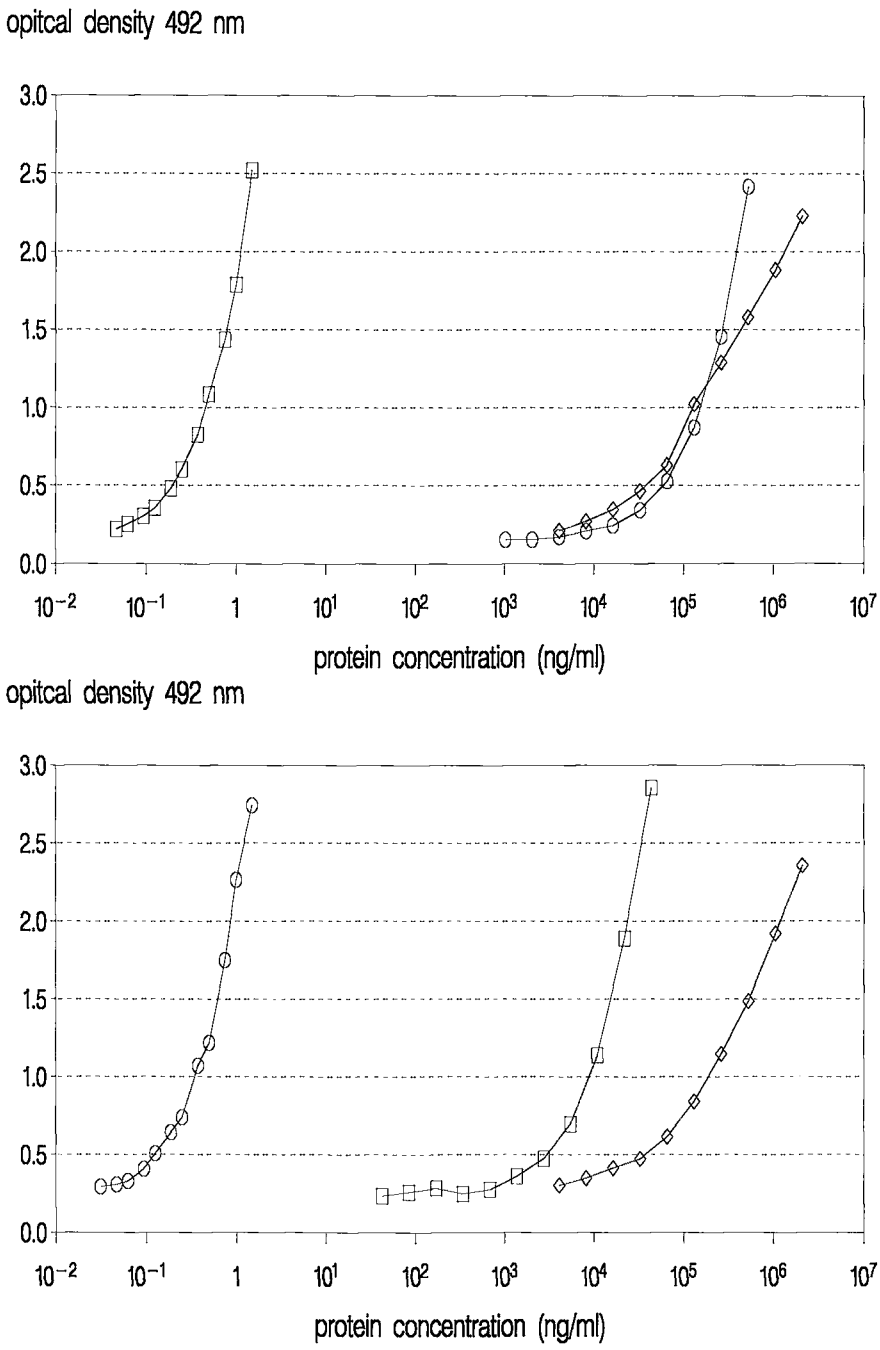


Figure 3. *Reactivity of RUA (□), MUA (○) and animal feed (◇) in the sandwich EIA for RUA (top) and for MUA (bottom).*

JOB-DEPENDENT ALLERGEN EXPOSURE

A total of 287 personal full-shift inhalable dust samples were collected. Forty-four percent of all samples yielded dust levels which were below the detection limit of inhalable dust. Of these 287 personal full-shift air samples 116 were collected during work with only rats, 36 during work with only mice and 135 during work with rats as well as mice. In 31% and 13% of the samples the RUA and MUA concentrations were below the detection limit, respectively. The inhalable dust and aeroallergen concentrations were moderately correlated ($r = 0.44$, $p < 0.01$ for RUA exposure; $r = 0.17$, $p = 0.024$ for MUA exposure). In general, dust concentrations were low and showed little variation over time and between jobs (range 0.11 - 0.64 mg/m^3), whereas the variability of the aeroallergen concentrations was large.

The median, 25th and 75th percentiles and range of the RUA and MUA exposure level distributions by job title are shown in figure 4. The highest personal exposure levels of RUA over a shift were measured for animal caretakers (median = 1.6 ng eq/m^3). Animal technicians and supervisors had almost similar median RUA exposure levels of 0.77 and 0.65 ng eq/m^3, respectively. For the scientists and scientific assistants more than half of the samples were below the detection limit. For MUA the highest exposure levels were found for the animal technicians (median = 12.1 ng eq/m^3) and caretakers (median = 6.4 ng eq/m^3), and the lowest exposure levels were found for the supervisors (median = 0.58 ng eq/m^3). For the scientists only two air samples have been collected during work with mice. The median MUA level of 2.7 ng eq/m^3 in this group is therefore not a representative value. The large differences between 25th and 75th percentiles and the large ranges of the RUA and MUA exposure levels illustrate the large variation of exposure within each job title group.

TASK-DEPENDENT ALLERGEN EXPOSURE

Reasons for the large variation of exposure within each job title group could be the differences in duration of exposure to rats or mice and/or differences in tasks performed at the day of sampling. To examine the effect of different tasks a total of 278 personal inhalable dust samples were collected during performance of defined tasks (table 1). The mean and range of the sampling time of each task is also described in table 1. Of the 278 personal task air samples 155 were collected during work with only rats, 82 during work with only mice and 41 during work with rats as well as mice. In 62% and 18% of the samples RUA and MUA could not be detected, respectively (table 2). Cleaning out cages and changing rats into new cages were associated with the highest exposure to RUA, GM of 5.6 and 5.3 ng eq/m^3, respectively. During feeding, which also disturbs the rats, and during the handling of rats, lower RUA

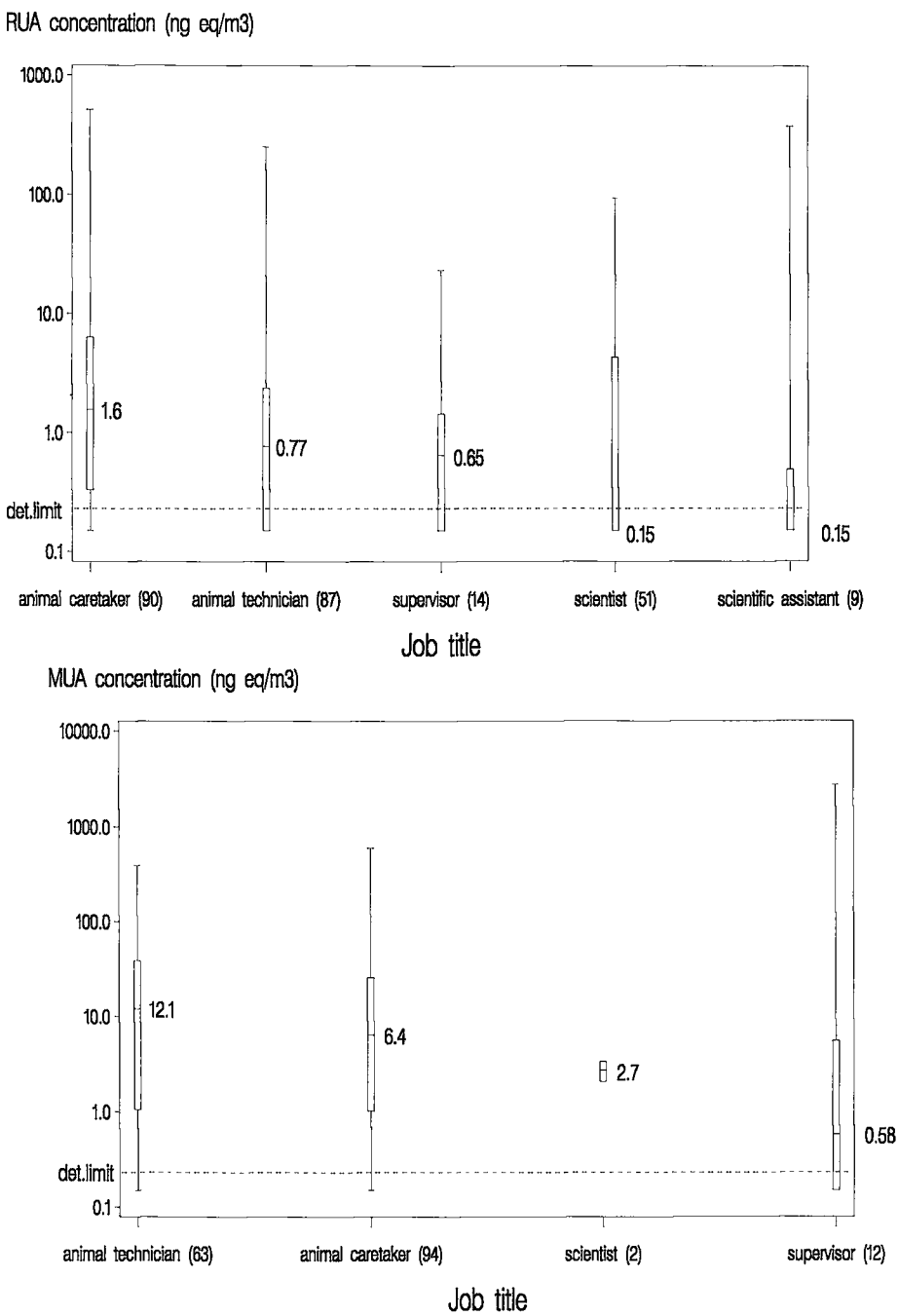


Figure 4. *Median (centre of box), 25th and 75th percentile (borders of box) and range (whiskers) of RUA (top) and MUA (bottom) concentrations (ng eq/m^3), stratified by job title (N = 251 for RUA exposure, N = 171 for MUA exposure).*

levels were found, GM of 2.4 and 1.2 ng eq/m^3, respectively. During all other tasks only a few samples had detectable levels of RUA. Tasks which involved handling of contaminated bedding or large numbers of conscious mice were associated with the highest exposure to MUA (GM = 16.0 - 74.8 ng eq/m^3). In addition, during feeding and during experiments with conscious mice, high concentrations of MUA (GM = 19.6 - 33.5 ng eq/m^3) were found. Tasks entailing work with unconscious or dead mice and indirect contact, like cage washing and cleaning rooms, were associated with low MUA exposure.

The GSD of the RUA and MUA levels of the tasks (table 2) were all above two, which indicates considerable variation in exposure within each task[85]. We therefore examined whether the differences in exposure level could be due to differences between the sites, i.e. the laboratory where samples were taken. For one task, cleaning out cages, the RUA and MUA exposure levels were further stratified by site (table 3). The highest concentrations were found at site C for RUA exposure (158 ng eq/m^3) and at site D for MUA exposure (2000 ng eq/m^3). These levels were extremely high when compared to the sites with low RUA levels (sites A, B, D and F, GM = 1.1 - 3.8 ng eq/m^3), or MUA levels (site B and E, GM = 0.63 - 21.2 ng eq/m^3). Despite the low number of samples at some sites, the sampling site appeared to be an important determinant of exposure.

DISCUSSION

Laboratory animal work usually consists of many different short lasting tasks and intermittent contact with laboratory animals. A thorough assessment of aeroallergen exposure in this environment requires a sensitive aeroallergen assay to account for this diversity. This paper describes two sensitive immunoassays with which rat and mouse urine allergens can be quantitatively detected in personal samples of airborne dust.

In the immunoassays, urinary proteins of rats and mice were used as reference preparations. Urinary proteins are very allergenic[45-49] and may comprise the most important allergens found in airborne dust in laboratory animal facilities[46,49]. Extracts of fur or epithelia of rats and mice are also commonly used in in vivo or in vitro tests for rat or mouse allergy, but the allergenicity of these preparations is mainly due to the presence of proteins that are also found in urine or saliva[46-49].

Electrophoresis and immunoblotting revealed a reaction profile of IgE from sensitised laboratory animal workers, which was very similar to that of the rabbit polyclonal antibodies used in the sandwich assays. The rat urine used in this study contained at least nine proteins, which all appeared to be allergenic. More than 50% of 56 sera from rat-sensitised workers showed reactions to the proteins with molecular weight

Table 2. *Geometric mean (GM), geometric standard deviation (GSD) and range of RUA and MUA airborne personal exposure levels (ng eq/m^3), stratified by task (N = 196 for RUA exposure, N = 123 for MUA exposure).*

	RAT					MOUSE				
task	N	Nd*	GM (ng eq/m^3)	GSD	range	N	Nd*	GM (ng eq/m^3)	GSD	range
cleaning out cages	29	12	5.6	9.8	0.63 - 1600	25	1	74.8	9.2	0.63 - 2700
changing into new cages	35	10	5.3	5.4	0.63 - 127	33	3	22.8	6.5	0.63 - 501
feeding	20	11	2.4	5.3	0.63 - 60	19	2	19.6	7.8	0.63 - 542
handling animals	34	22	1.2	3.7	0.63 - 94	8	1	16.0	5.7	0.63 - 209
experiments 2	7	5	1.0	2.5	0.63 - 6.3	2	0	33.5	7.5	8.0 - 140
experiments 1	25	20	0.85	2.2	0.63 - 9.0					
biotechnical work	23	21	0.83	2.6	0.63 - 34	11	2	5.4	4.2	0.63 - 51
cage washing	8	7	0.81	2.1	0.63 - 4.9	8	4	2.6	6.4	0.63 - 89
cleaning rooms	15	13	0.80	2.2	0.63 - 14	17	9	2.4	5.6	0.63 - 151

Table 3. *Geometric mean (GM), geometric standard deviation (GSD) and range of RUA and MUA airborne personal exposure levels (ng eq/m^3) when cleaning out the cages, stratified by site.*

cleaning out cages	RAT					MOUSE				
site	N	Nd*	GM (ng eq/m^3)	GSD	range	N	Nd*	GM (ng eq/m^3)	GSD	range
A	6	2	2.5	3.6	0.63 - 17.4	6	0	50.3	3.7	11.7 - 246
B	9	7	1.1	2.9	0.63 - 9.3	10	0	21.2	3.5	4.8 - 168
C	6	0	158	3.5	45.9 - 1630	6	0	677	2.6	165 - 1520
D	2	1	3.8	>10	0.63 - 23.4	2	0	2000	1.5	1470 - 2710
E	4	2	2.3	4.5	0.63 - 10.5	1	1	0.63	-	-
F	2	0	38.4	2.3	21.6 - 68.1					

* Nd, number of samples below the detection limit.

66, 22 and 15 kd, which is consistent with findings in previous studies. These studies[45,49-51] have distinguished two 'major' rat urinary allergens, *Rat n* IA (20-21 kd) and *Rat n* IB (16-17 kd). Rat albumin (68 kd) also has been shown to possess some allergenicity[49,51,52], but has not been identified as 'major' rat urinary allergen. Similarly, all detectable proteins in mouse urine reacted with IgE antibodies from sensitised workers. An allergenic reaction was also found to a component with molecular weight of 40 kd, which could not be detected by Coomassie staining. More than 50% of 47 sera of mouse-sensitised workers showed reaction to the proteins with molecular weight 40, 22 and 18 kd. This is also consistent with other studies[46,50,53-55] in which two 'major' mouse urinary allergens have been found, *Mus m* I (17-21 kd) and *Mus m* II (16 kd). The allergen with a molecular weight of 40 kd has however not been described previously.

In immunoblotting some cross-reactivity was found between anti-MUA antibodies and two rat urinary proteins (15 kd and 66 kd). However, in the sandwich EIA for mouse urine the RUA activity was 25,000 times less than that of MUA. The rat urine assay showed to be even more specific. Animal feed, which may also be present in the workplace simultaneously, is not very likely to be a disturbing factor in the assays. All personal dust samples were below the 0.64 mg/m^3. If this dust level was totally due to animal feed, and taking into account that the protein content of animal feed is approximately 4%, this concentration would give OD_{492} values that would hardly differ for the background (figure 3). Therefore, cross-reactivity with proteins which could be simultaneously found in rat and mouse rooms will be of minor importance in both assays.

The EIAs for rat and mouse urine were also highly sensitive and reproducible. The detection limit was about 0.075 ng eq/ml, equivalent on average to 0.23 ng eq/m^3 for full-shift and 0.94 ng eq/m^3 for task samples. The reproducibility of the assays was comparable to that reported in other studies[58,63]. The rabbit anti-RUA and anti-MUA antibodies as well as rat and mouse urinary allergens were obtained in large quantities to ensure continuity of the assays for years.

The rabbit antibodies reacted with all allergens present in rat and mouse urine, respectively. Thus, using these polyclonal antibodies in immunoassays may have the advantage of measuring the relevant exposure, i.e. all potential allergens, even if the composition of dust differs in time and place, for example due to differences in sex and age of the animals[49]. Monoclonal antibodies, measuring only one 'major' allergen, may fail to detect high concentrations when relatively more of the other allergens are present in dust. Still, monoclonal antibodies have the advantage of offering a better standardisation of the assay, because only one well defined allergen is measured. We

therefore preferred to express the allergen concentrations in ng protein *equivalent* per m^3, since two samples with the same level of allergenic reactivity in the assay, might contain a different composition of RUA or MUA. Equivalent was also used, because allergen levels presented in other studies have been measured by assays using different reference preparations, like one 'major' urinary allergen[61,62], or different types of antibodies, like antibodies derived from sensitised workers[60,62,63] or monoclonal antibodies[61]. Further, differences could be found in type of assay used, like an inhibition assay[60,62,63], and in dust sampling equipment or extraction method. Therefore, the absolute values of allergen concentrations reported by different studies should be compared with care. A recent study[64] has compared two different assays to measure RUA and concluded that the inhibition IgE-assay[63] gave concentrations which were several orders of magnitude higher compared to the concentrations found in the sandwich monoclonal assay, although the concentrations were highly correlated ($R^2 = 0.72$). Preliminary results of a study in which both assays are compared with our assays also reveal very good correlations between the assays. The concentrations measured by the inhibition IgE-assay are again several orders of magnitude higher compared to the concentrations found with the other two assays[in preparation]. Thus, exposure measurements may be compared by the use of conversion factors, because of the high correlations found between the measurement methods, although eventually a thorough standardisation is of course to be preferred.

As shown previously[63,67] animal caretakers experienced the highest exposure to RUA. For MUA the caretakers as well as the animal technicians had the highest exposure. The large variability in exposure within each job title was striking and indicates that information regarding the job performed is probably not sufficient to be used as a measure of exposure in epidemiological studies. This variability appeared to be partly due to the wide range of tasks performed (table 2). Tasks which involved handling contaminated bedding or conscious rats and mice were associated with the highest exposure levels, which is in agreement with previous reports[58,62,67]. This may be useful information for both occupational hygiene and epidemiological studies, in which the time spent performing high exposed tasks may be important. However, there remained a large variability in allergen concentrations within each task. Some of this variability could be explained by differences between sites (table 3). These differences in exposure levels may be due to the level of contamination of the bedding in cages which are cleaned, varying numbers of animals of different sex and age used during different tasks, speed with which tasks are performed, and type and varying levels of ventilation in rooms in which tasks are performed. These factors underline the complexity of exposure assessment for laboratory animals workers. A paper

describing the determinants of RUA and MUA exposure in more detail is in preparation.

This study shows that the rat and mouse urinary allergen assays are very sensitive and are appropriate means of quantifying low levels of aeroallergens in personal air dust samples. The exposure assessment of RUA and MUA thus allows the identification of sources of exposure as well as the study of exposure-response relationships.

CHAPTER 3

DETERMINANTS OF AIRBORNE RAT AND MOUSE URINARY ALLERGEN EXPOSURE

ABSTRACT

Background: Several studies have shown that the risk of developing laboratory animal allergy is related to the allergen exposure level.

Objective: Determination of the factors affecting exposure to rat and mouse urinary allergens.

Methods: Ambient and personal air sampling was performed in 7 animal facilities (sites).

Results: The ambient air samples showed that the number of animals present in the room explained more than half of the variability in allergen concentration. The allergen concentration increased approximately 1.7 times when the number of animals was doubled. The allergen levels were twice as high on Mondays compared to the other days, which was due to specific high exposure tasks. The filter top cages reduced the allergen levels between 6 and 17 times. An inverse day/night rhythm (infrared light) resulted in 11 times higher rat allergen levels.

The highest personal exposure levels were found during handling of contaminated bedding and handling of high numbers of conscious rats or mice. The proportion of time spent on these tasks determines exposure to the allergens to a large extent. The average ambient air allergen level in the animal room probably plays a minor role. Finally, personal exposure to rat and mouse urinary allergens differed considerably between facilities.

Conclusions: The number of animals, filter top cages, and infrared lights were important factors of ambient air rat and mouse allergens levels in animals rooms in Dutch facilities. The personal rat and mouse allergens exposure was predominantly determined by task performed and site. The presented determinants can be used for further study on exposure reduction and laboratory animal allergy.

---

by Albert Hollander, Dick Heederik, Gert Doekes and Hans Kromhout, submitted for publication

INTRODUCTION

Laboratory animal workers are at high risk of developing occupational allergy[12-14,17,18,22,34,83]. Several studies[18,22,86] have shown that the risk of developing laboratory animal allergy (LAA) is related to the allergen exposure level. Exposure control can potentially decrease the risk of developing LAA. Effective exposure control, however, requires detailed knowledge of the most important determinants of exposure[87,88]. When available. this knowledge may be used to estimate exposure levels in epidemiological studies[89-91].

Rat and mouse urinary proteins are highly allergenic[45-49] and are the most important allergens in airborne dust in laboratory animal facilities[46,49]. In previous studies associations have been found between ambient air levels of urinary allergens and number of animals in the room[68,92,93], bedding material used[68,94], relative humidity in rooms[95], use of filter top cages[68] and rate of ventilation[96]. Little is known, however, about the determinants of personal allergen exposure levels of laboratory animal workers.

In the Netherlands a large study among approximately 600 laboratory animal workers started in 1992. The aim was to study relationships between aeroallergen exposure and LAA. As part of this epidemiological study on LAA, ambient air sampling in animal rooms, and shift and task-based personal air sampling was performed. This paper describes an analysis of potential determinants of rat (RUA) and mouse (MUA) urinary aeroallergen exposure. Linear regression models were used to unravel the factors affecting RUA and MUA concentrations. In contrast to other studies[68,93,97], air samples were taken at more than one laboratory animal facility. Furthermore, this study had no experimental design like most earlier studies[68,94-96], but examined determinants of exposure to RUA and MUA under normal working conditions.

MATERIALS AND METHODS

SITES

Seven laboratory animal facilities, which were affiliated to universities (sites A, B, C and D), non-university research institutes (sites E and F) and a pharmaceutical company (site G), took part in this study. In these 7 sites, 21 different exposure zones could be distinguished. A zone was defined on the basis of the working environment, i.e. similarity in ventilation system, construction of building or equipment[98]. In site A and site G only one zone could be distinguished, in site D and F two zones, in site E three zones, in site C five zones, and in site B seven zones (table 1).

Among laboratory animal workers five broad job titles could be distinguished: *Animal caretakers*, who are involved in 'general care of animals' and cleaning of cages and

rooms; *Animal technicians*, who carry out experimental procedures on animals on a full-time basis; *Scientists* and *scientific assistants*, who perform experiments on animals, their tissues or body fluids, on a part-time basis. In general, the scientific assistants perform the routine experiments; *Supervisors*, who have no direct contact with animals or their tissues or body fluids, but are regularly present in the laboratory animal rooms.

Table 1. Characteristics of the animal rooms of the participating sites

site	no.of zones	no. of rooms sampled	range no. of rats/room	range no. of mice/room	range temperature (°C)	range rel. humidity (%)	range ventilation rate (air changes/hour)
A	1	2	95 - 200	---	21 - 21	56 - 56	34 - 36
B	7	12	45 - 177	250 - 1000	19 - 25	37 - 75	10 - 36
C	5	8	22 - 150	435 - 900	21 - 24	45 - 70	18*
D	2	8	100 - 196	190 - 350	21 - 24	---	10 - 22
E	3	6	200 - 416	48 - 475	20 - 23	42 - 70	27 - 32
F	2	5	45 - 73	115 - 360	21 - 25	45 - 73	27 - 34
G	1	4	25 - 122	25 - 108	21 - 22	---	16 - 22

* the inlet as well as the outlet of the ventilation system was not accessible. The ventilation rate was obtained from the technical services department of the facility.

AIR SAMPLING

To determine the particle size distribution of the allergen carrying dust, measurements were performed with an Andersen cascade impactor (Andersen, Atlanta, GA, USA). The sampler consists of eight stages and a backup. The flow rate was 28.3 l/min. The sampler was placed close to the animals. In total 10 samples were collected during full-shift periods of 6-8 hours, using glass fibre filters (Whatman GF/A).

Total dust ambient air sampling was carried out at fixed points close to the animals at an average height of 1.5 m. The concentration of total dust was determined using a modified Schleicher and Schüll PL050/1 sampling head with a face velocity of 1.25 m/s in the inlet opening and a flow rate of 23.5 l/min. In total 152 dust samples were collected during full-shift periods of 6-8 hours, using polytetrafluoroethylene (Teflon) filters (Millipore; pore size 1.0 µm, Ø 4.5 cm). At time of sampling the number of animals in the room was counted, temperature and relative humidity were measured, and type of cage and bedding material were recorded. Furthermore, the ventilation rate (air changes per hour) was determined by measuring the inlet or outlet air velocity (Heat wire anemometer, Gill 1012 R2). The ranges of these variables are presented in table 1.

Personal inhalable dust was sampled using IOM sampling heads[99] with a flow rate of 2

l/min. Each sampling head contained a polytetrafluoroethylene (Teflon) filter (Millipore; pore size 1.0 μm, ∅ 2.5 cm). Two hundred eighty seven samples were collected during shifts of 4-8 hours. The samples were taken in a random sample of the workers. During sampling the workers had to record tasks performed on a checklist. Each worker was sampled for one week on days when he or she was working in the laboratory animal facility.

In addition, 278 personal samples were collected during various tasks, which were defined in advance (table 2). Samples were taken only when the task was actually being performed. Dust sampling was carried out at all sites between September 1992 and November 1993.

Table 2. *Task categories in laboratory animal work. The number of samples (working with rats and/or mice) and sampling time were presented as well.*

TASK CATEGORIES	
1 cleaning rooms	cleaning rooms and sweeping zones (mean sampling time 58 minutes, range 10 - 128, rat n=15, mouse n=17)
2 change into new cages	changing animals from dirty into clean cages & cleaning racks (mean sampling time 73 minutes, range 10 - 270, rat n=35, mouse n=33)
3 cleaning out cages	removing woodchips from dirty cages (mean sampling time 56 minutes, range 7 - 218, rat n=29, mouse n=25)
4 cage washing	bottle and cages washing in cage washing area (mean sampling time 64 minutes, range 16 - 112, rat n=8, mouse n=8)
5 feeding	changing animal feed and water bottles (mean sampling time 57 minutes, range 5 - 186, rat n=20, mouse n=19)
6 handling animals	handling of animals in animal room, for example tumour control, weighing, taking temperature (mean sampling time 85 minutes, range 10 - 341, rat n=34, mouse n=8)
7 biotechnical work	immunisation, injection, blood sampling (mean sampling time 92 minutes, range 15 - 325, rat n=23, mouse n=11)
8 experiments 1	experimental work on anaesthetised animals (mean sampling time 163 minutes, range 25 - 317, rat n=25)
9 experiments 2	experimental work on conscious animals; handling/observing (mean sampling time 161 minutes, range 85 - 267, rat n=7, mouse n=2)

ELUTION AND ANALYSIS OF RUA AND MUA

RUA and MUA were recovered from the filters by extraction at room temperature with 2 ml (inhalable dust samples), 4.5 ml (total dust samples) or 15 ml (Andersen samples) phosphate buffered saline (PBS, Ph 7.4). Filters were successively vortexed for 2 minutes, sonicated for 2 minutes, vortexed for 5 minutes and sonicated for 2

minutes. The extract was centrifuged at 5,000 g for 15 minutes and the supernatant was collected and stored at -20°C. The extracts of Andersen samples (glass fibre filters) were centrifuged twice.

RUA and MUA were assayed by sandwich enzyme immunoassay. Details of analysis of RUA and MUA are reported elsewhere (Chapter 2)[100]. Urine from Wistar rats and Balb/c mice were used as standard preparations in the immunoassays. Concentrations in test samples were expressed in ng urinary protein equivalent (ng eq) per ml in which 1 ng eq was defined as the amount giving the same assay result as 1 ng protein of the standard.

The detection limit was estimated by analysing extracts of 126 blank filters. These filters underwent the same procedures as the loaded filters except for actual air sampling. The average allergen concentrations estimated in these extracts was 0.026 ng eq/ml (SD = 0.028) for the RUA assay and 0.018 ng eq/ml (SD = 0.023) for the MUA assay. Only extracts of air samples with a concentration higher than the mean concentration of these blanks + 2 SD were considered positive, which implied a detection limit of 0.075 ng eq/ml for the RUA as well as the MUA assay. Due to differences in sampling time and flow rate the detection limits were 0.030 ng eq/m^3 for ambient air total dust samples, 0.23 ng eq/m^3 for personal full-shift samples and 0.94 ng eq/m^3 for personal task samples. Two third of these detection limits were assigned to samples in which RUA or MUA were below the limit of detection.

STATISTICAL ANALYSIS

All collected environmental factors were included in classical linear regression models (SAS, version 6.09, procedure GLM) in order to unravel factors affecting ambient air and personal RUA and MUA concentrations. Subsequently, the determinants which were not statistically significant ($p < 0.1$) were excluded from the model. In the analyses, the logarithm of the RUA and MUA concentration was used in order to stabilise the variance. Continuous, e.g. the number of animals, as well as dummy variables, e.g. the use of filter top cages (yes/no), were used as independent variables in the empirical models. The logarithm of the number of animals was used in order to obtain a linear relationship with the RUA and MUA concentrations.

RESULTS

PARTICLE DISTRIBUTION

Table 3 shows the size distribution of the dust particles carrying allergens. The amount of allergen captured in each stage has been expressed as percentage of the total allergen concentration. The RUA were carried on particles larger than 5.8 μm diameter and 78%

percent of all allergens was found on particles with a diameter larger than 9 μm. The majority of MUA was also found on particles larger than 5.8 μm diameter (72%). However, 24% was found on smaller particles with a diameter between 2.1 and 5.8 μm.

Table 3. *Mean and ranges of the distribution of allergen particles, presented as a percentage of the total urinary aeroallergen concentration.*

	RAT (n = 4)		MOUSE (n = 6)	
aerodynamic diameter (mm)	mean (%)	range	mean (%)	range
> 9.0	78	62 - 100	47	22 - 64
5.8 - 9.0	22	0 - 38	25	20 - 33
4.7 - 5.8	0	---	8	1 - 13
3.3 - 4.7	0	---	8	1 - 16
2.1 - 3.3	0	---	8	1 - 25
< 2.1*	0	---	4	0 - 6
total allergen conc (ng eq/m^3)	0.57	0.13 - 0.90	14.8	3.6 - 37.8

* four stages of the Andersen sampler were grouped

AMBIENT AIR SAMPLING

Ambient air dust sampling was performed in 16 of the 21 zones in a total of 40 rooms (table 1). The remaining 5 zones were not accessible for our ambient air sampling equipment, because of possible contamination of the animals. The average number of animals was 128 (range 22 - 416) in rat rooms and 404 (range 25 - 1000) in mouse rooms. At site C filter top cages were used in one mouse and one rat room. In one rat room at site G infrared lights were used in order to achieve an inverse day/night rhythm. The ventilation rate in all animal rooms was in between 10 - 25 air changes per hour, except for the animal rooms at site A, $B_{zone\ 6}$, $E_{zone\ 2}$, and F where the ventilation rate was more than 25 air changes per hour. Furthermore, the average temperature of the air in the room was 22°C (range 19°C- 25°C) and the average relative humidity was 58% (range 37% - 75%).

Of the 152 ambient air total dust samples 83 were collected in rat rooms, 61 in mouse rooms and 8 in rooms in which rats as well as mice were present. The median, 25th and 75th percentiles and range of the RUA and MUA levels in rat as well as mouse rooms are shown in figure 1. The median RUA concentration in rat rooms was 0.90 ng eq/m^3(figure 1 top). Compared to the median RUA concentration in rat rooms, the median MUA concentration in mouse rooms was higher, 7.2 ng eq/m^3 (figure 1 bottom). Additionally, 51% of the samples in rat rooms had no detectable MUA level and in mouse rooms RUA could not be detected in 85% of the air samples.

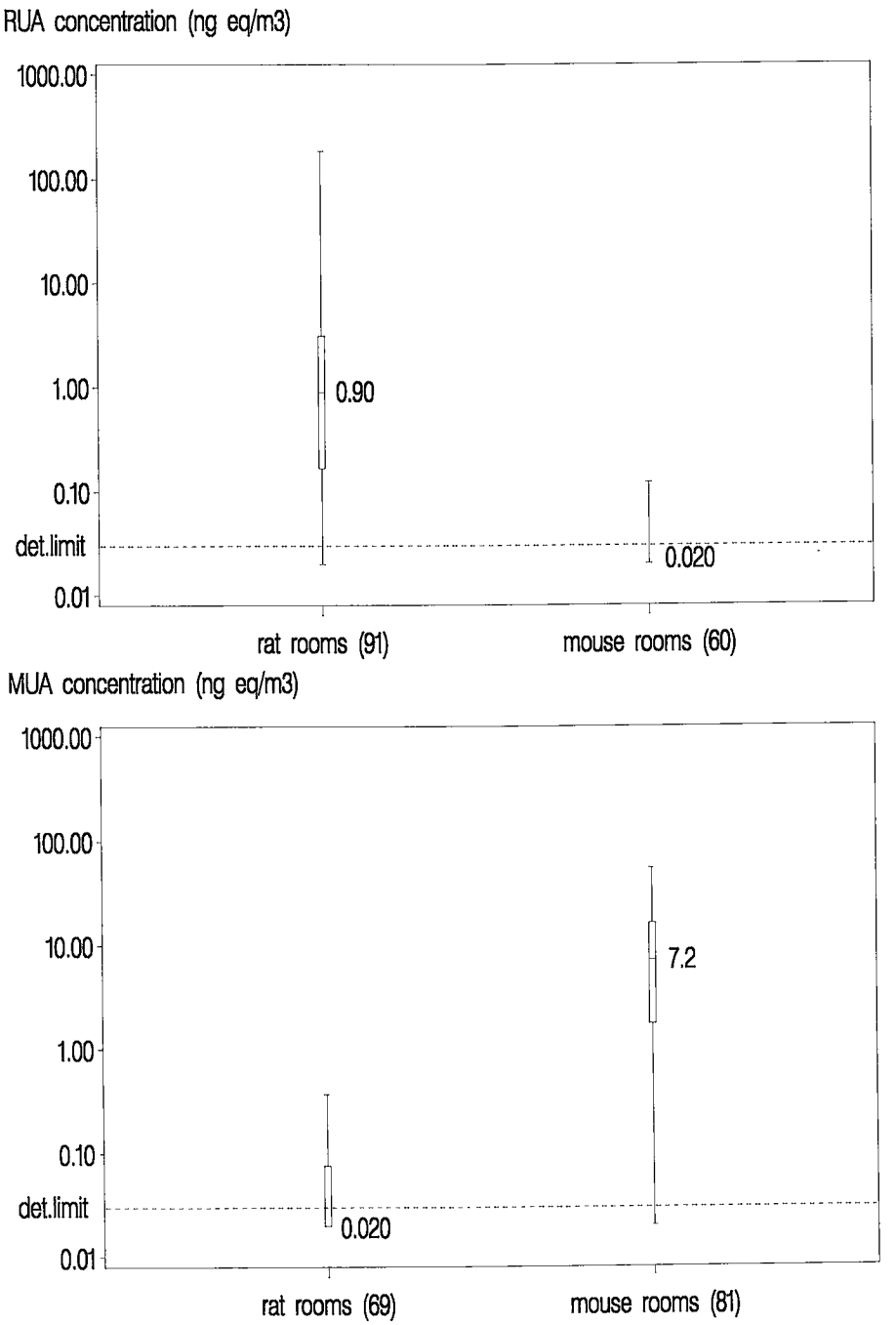


Figure 1. *Median (centre of box), 25th and 75th percentile (borders of box) and range (whiskers) of RUA (top) and MUA (bottom) concentrations (ng eq/m^3), stratified by type of animal room.*

Figure 1 illustrates the large variability in RUA and MUA levels found in rat and mouse rooms. Regression analysis revealed that the logarithm of number of animals in the room explained most of the variability in the logarithm of RUA and MUA concentration. Other determinants which contributed significantly to the models were the use of filter top cages, use of infrared lights, and day of the week the sample was taken (tables 4 and 5). The presented models explained 66% and 80% of the variability of ambient air RUA and MUA levels, respectively. No additional variability of RUA and MUA levels could be explained by the other variables, such as volume, ventilation rate, temperature and humidity of the room.

Table 4. *Analysis of variance model used to estimate the RUA concentrations in relation with various animal room characteristics.*

outcome variable: log10 (RUA concentration)						
source	df	SS	MS	F	p	R^2
model	4	97.4	24.4	70.6	0.0001	0.659
error	146	50.4	0.35			

characteristics	df	type III SS	F	p	ß* (SE)
log(number of rats in room)	1	79.0	229	0.0001	0.72 (0.05)
Monday versus Tuesday-Friday	1	1.3	3.8	0.0519	0.26 (0.13)
filter top on cages	1	4.5	13.1	0.0004	-0.77 (0.21)
infrared light in room†	1	4.2	12.2	0.0010	1.05 (0.30)

RSD = 0.58

* to estimate the geometric mean for a set of conditions, the background level (0.023 ng eq/m^3) should be multiplied with $10^{(ß * factors)}$; e.g. 80 rats in open cages with normal lights will give an estimated RUA concentration on a Monday of $10^{(0.72 * \log(80) + 0.26 * 1 - 0.77 * 0 + 1.05 * 0)} \times 0.023 = 0.98$ ng eq/m^3.

† infrared lights were used in order to achieve an inverse day/night rhythm.

The regression models revealed that doubling the number of rats or mice would increase the RUA or MUA levels 1.6 and 1.8 times, respectively. In addition, in rooms with filter tops on the cages the RUA and MUA levels were approximately 6 and 17 times lower compared to the levels found in similar rooms without filter tops on the cages. The RUA and MUA levels were approximately twice as high on Mondays compared to the other days of the week. Furthermore, in rat rooms with an inverse day/night rhythm, i.e. using infrared lights, the RUA levels were about 11 times higher than the RUA levels found in normal rat rooms. An inverse day/night rhythm was not present in the mouse rooms of the participating facilities.

Table 5. *Analysis of variance model used to estimate the MUA concentrations in relation with various animal room characteristics.*

outcome variable: log10 (MUA concentration)						
source	df	SS	MS	F	p	R^2
model	3	163.9	54.6	199.4	0.0001	0.804
error	146	40.0	0.27			

characteristics	df	type III SS	F	p	ß* (SE)
log(number of mice in room)	1	153.6	560	0.0001	0.82 (0.03)
Monday versus Tuesday-Friday	1	2.6	9.6	0.0023	0.36 (0.11)
filter top on cages	1	11.5	42.0	0.0001	-1.24 (0.19)

RSD = 0.52

* to estimate the geometric mean for a set of conditions, the background level (0.040 ng eq/m3) should be multiplied with $10^{(ß * factors)}$; e.g. 290 mice in open cages will give an estimated MUA concentration on a Monday of $^{10(0.82 * \log(290) + 0.36 * 1 - 1.24 * 0)}$ x 0.040 = 9.6 ng eq/m3.

PERSONAL SHIFT SAMPLING

A total of 287 personal shift inhalable dust samples were collected. Of these samples 116 were collected during work with only rats, 36 during work with only mice and 135 during work with rats as well as mice. The median RUA and MUA concentration of all samples were 0.76 ng eq/m^3 and 6.4 ng eq/m^3, respectively. The variability in RUA and MUA exposure was large with a 175 and 1400 fold difference between the 5th and 95th percentiles of the distributions, respectively. Of the large variability in both RUA and MUA concentration 22% was explained by job title, site and day of the week the sample was taken (tables 6 and 7). The regression models revealed that animal caretakers had the highest RUA exposure. However, job title was not significantly related to the variability in MUA exposure. Site explained most of the variability in RUA and MUA exposure giving a 100 and 23 fold difference in estimated RUA and MUA exposure between the sites with the lowest and highest exposure. Additionally, the RUA and MUA levels of the personal shift samples were higher on Mondays than on the other days of the week, 2.3 and 4.5 times respectively.

The large variation of RUA and MUA exposure within each job title group may be due to the differences in duration of exposure to rats or mice and/or differences in tasks performed at the day of sampling. Table 8 shows the average proportion of time spent on the various tasks, during contact with rats or mice, or with tissues, faeces or urine from rats or mice. Animal caretakers were working on average 27 hours per week with rats or mice. When working with laboratory animals, animal caretakers spent approximately 80% of their time on feeding, cleaning room/cages and changing animal to new cages. In

contrast, animal technicians, scientists and scientific assistants were working fewer hours per week with mice or rats, 16, 5 and 5 hours respectively. In addition, their work with laboratory animals consists for more than 50% of handling animals, biotechnical work, and experiments on anaesthetised animals. Normally, supervisors have no direct contact with animals, but due to leave of absence of other workers they had to take over their tasks occasionally, i.e. on average 3 hours per week.

Table 6. Analysis of variance model of the personal shift RUA concentrations.

outcome variable: log10 (RUA concentration)						
source	df	SS	MS	F	p	R^2
model	11	36.2	3.3	6.1	0.0001	0.22
error	239	128.2	0.54			
characteristics	df	type III SS		F	p	ß* (SE)
job title	4	6.6		3.1	0.018	
animal caretaker						0.38 (0.24)
animal technician						0.02 (0.26)
scientific assistant						-0.33 (0.33)
scientist						0.17 (0.24)
supervisor						0
site	6	23.7		7.4	0.0001	
A						-0.54 (0.94)
B						0.60 (0.57)
C						0.21 (0.57)
D						1.02 (0.57)
E						0.55 (0.59)
F						1.46 (0.60)
G						0
Monday versus Tuesday-Friday	1	4.4		8.3	0.0045	0.37 (0.13)

RSD = 0.72

* to estimate the geometric mean for a given set of conditions, the background level (0.15 ng eq/m^3) should be multiplied with $10^{(ß * factors)}$; e.g. an animal caretaker at site B will have an estimated RUA concentration on a Monday of $10^{(0.38 + 0.60 + 0.37 * 1)} \times 0.15 = 3.4$ ng eq/m^3.

PERSONAL TASK SAMPLING

A total of 278 task-specific personal inhalable dust samples were collected during performance of defined tasks in six of the seven sites. Forty six shift samples, where work entailed only one task, were included in these 278 task samples. The mean and range of the sampling time of each task was also described in table 2. Of the 278

personal task-specific air samples 155 were collected during work with rats, 82 during work with mice and 41 during work with rats as well as mice. Respectively, 62% and 18% of the samples had no detectable RUA and MUA levels. The median, 25th and 75th percentiles and range of the RUA and MUA levels of the tasks are shown in figure 2. Changing rats into new cages and cleaning out cages were associated with the highest exposure to RUA, median of 6.7 and 5.5 ng eq/m^3, respectively. During all other tasks the median RUA levels were below the detection limit. Tasks which involved handling of contaminated bedding or experiments with large numbers of conscious mice were associated with the highest exposure to MUA; median levels of 73 and 74 ng eq/m^3, respectively. In addition, during changing mice into new cages, handling of mice, feeding, and biotechnical work intermediate levels of MUA were found, median 23, 22, 15 and 7.4 ng eq/m^3 respectively. Tasks entailing indirect contact with mice, like cage washing and cleaning rooms, were associated with the lowest MUA exposure.

Table 7. Analysis of variance model of the personal shift MUA concentrations.

outcome variable: log10 (MUA concentration)						
source	df	SS	MS	F	p	R^2
model	9	36.7	4.1	5.1	0.0001	0.22
error	161	129.5	0.80			
characteristics	df	type III SS		F	p	ß* (SE)
job title	3	0.92		0.4	0.77	
animal caretaker						0.31 (0.30)
animal technician						0.33 (0.33)
scientific assistant						---
scientist						0.39 (0.70)
supervisor						0
site	5	19.7		4.9	0.0003	
B						1.3 (0.95)
C						0.53 (0.95)
D						0.93 (0.95)
E						1.4 (0.97)
F						-0.02 (1.1)
G						0
Monday versus Tuesday-Friday	1	11.6		14.4	0.0002	0.65 (0.17)

RSD = 0.87

* to estimate the geometric mean for a given set of conditions, the background level (0.15 ng eq/m^3) should be multiplied with $10^{(ß * factors)}$; e.g. an animal caretaker at site B will have an estimated RUA concentration on a Monday of $10^{(0.31 + 1.3 + 0.65 * 1)} \times 0.15 = 27.3$ ng eq/m^3.

Table 8. *Percentage* of time spent on various tasks during contact with rats or mice, or with tissues, faeces or urine from rats or mice and hours per week working with laboratory animals.*

job title	task category (see table 2)								hours/week working with animals †
	1	2	3	4	5	6	7	8	
animal caretaker	19	2	19	14	24	8	2	2	27
animal technician	10	<1	7	5	17	10	33	12	16
scientific assistant	6	9	<1	<1	3	4	9	47	5
scientist	<1	2	<1	<1	3	29	19	40	5
supervisor	22	<1	6	14	15	17	4	<1	3

* does not add up to 100% for each job title, because a small percentage is spent on miscellaneous tasks

† derived from the questionnaire distributed among all workers of the participating facilities

Regression analysis revealed that task, site and the interaction between task and site explained 56% and 51% of the variability in RUA and MUA levels, respectively (RUA: F=10.5, df=21, p=0.001, RSD=0.48; MUA: F=5.6, df=19, p=0.001, RSD=0.67). In these analyses the tasks feeding and handling animals were grouped as well as the tasks room/cages washing, biotechnical work and experiments. This resulted in a total of four tasks. Cleaning out cages was associated with the highest RUA and MUA exposure. However, interaction between site and task explained a large amount of the variability in RUA and MUA exposure, approximately 20% in both models.

DISCUSSION

Large differences in ambient air RUA and MUA concentrations were found when various animal rooms were compared. This variability in allergen concentration could be explained for more than half by the number of animals in the room. An increase of the allergen concentration with increasing number of animals was consistent with other exposure studies of RUA[68,93] and MUA[92]. The RUA and MUA level increased approximately 1.6 and 1.8 times when the number of rats or mice was doubled, respectively. As a result, the estimated RUA concentration was approximately 8 times higher in a rat room housing the maximum number of rats (416) compared to a similar rat room housing the minimum number of rats (22). For mouse rooms this difference was even larger, approximately 14 times.

The activity of the animals has a large effect on the ambient air allergen level in rooms. The inverse day/night rhythm, which makes rats more active during working hours, resulted in 11 times higher RUA levels in the rooms. This is consistent with findings in other studies, in which the aeroallergen concentration in rooms increased when the animals were disturbed[61,94,97].

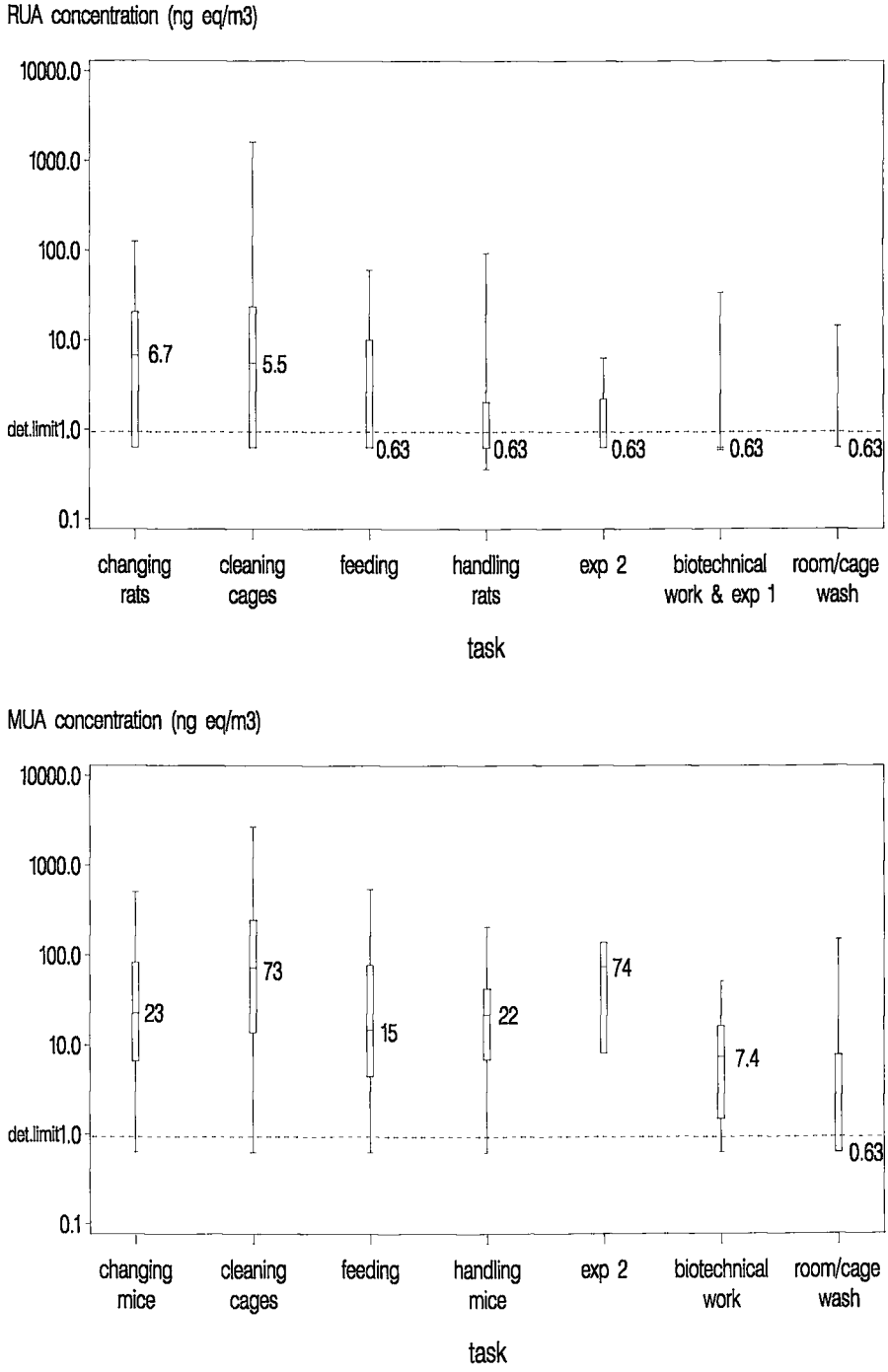


Figure 2. *Median (centre of box), 25th and 75th percentile (borders of box) and range (whiskers) of RUA (top) and MUA (bottom) concentrations (ng eq/m^3), stratified by task.*

Our study showed that use of filter top cages may be an appropriate control measure to reduce ambient air allergen level in the rooms. In rat and mouse rooms where filter tops were used, the RUA and MUA concentrations were 6 and 17 times lower compared to similar rooms without filter top cages. This is consistent with another study[68], in which the ambient air RUA concentration was reduced by filter tops to levels similar to those found in the same room in the absence of rats.

On Mondays the ambient air RUA and MUA levels were significantly elevated compared to the levels on the other days of the week. In addition, the personal RUA and MUA exposure levels were also elevated on Mondays. On Mondays, approximately 70 percent of the time is spent on cleaning out dirty cages and changing animals into new cages. Furthermore, the animal caretakers had the highest personal RUA and MUA exposure. Animal caretakers spent the largest proportion of their work on cleaning out cages, changing animals into new cages, feeding and handling large numbers of conscious animals. These tasks, which could be summarised as working with contaminated bedding material or handling large numbers of conscious animals, were associated with the highest aeroallergen exposure[58,62,67]. Therefore, the proportion of time spent on these tasks will determine the RUA or MUA exposure of the workers to a large extent.

During the performance of tasks involving contaminated bedding material or handling large numbers of conscious animals the workers were highly exposed to animal allergens. However, considerable variability in allergen exposures occurred within task categories. Some of this variability could be explained by differences between and within sites. The substantial contribution of an interaction term in the models suggested that considerable differences existed between similar tasks performed at different sites. Possible important factors may be the determinants of ambient air levels, i.e. number of animals in the room, use of filter top cages, and use of infrared lights (tables 4 and 5). However, the influence of these factors on the variability in personal exposure levels could not be determined due to the fact that a large number of tasks (cleaning cages, cages washing, biotechnical work and the experiments) were not performed in the animal rooms. In addition, a worker often performed a task in more than one room. The animal density in these rooms could differ substantially. Other factors which may contribute to the differences in personal exposure levels which are due to site, are the level of contamination of the bedding material, personal behaviour of the worker, e.g. pace with which tasks are performed, and type and varying levels of local ventilation equipment or other exposure control equipment. However, these determinants were not investigated in our study.

The differences between sites could not be ignored and will be important for estimating

the RUA and MUA exposure levels of the workers. The large influence of site is similar to results found in a study in the rubber industry[87] and in granite sheds[89]. However, the large influence of site in our study is in contrast to another study in laboratory animal facilities[101], in which job title explained a much larger percentage of the variability in RUA levels than differences between sites. A possible explanation is the larger number of sites sampled in our study, seven versus only two in the study of Nieuwenhuijsen et al[101].

The empirical modelling as presented in this paper, has several limitations. A large proportion of the variance in personal RUA and MUA levels remained unexplained in the linear models, because of factors not accounted for in this study. In addition, evaluation is restricted to explanatory variables with sufficient variation. An important difference with other studies[68,93,97] is that our study was performed at different sites, which should account for sufficient variation in the various variables. Despite the large number of sites in our study, we could not find any association between ambient air allergen concentrations and rate of ventilation and relative humidity in the room, which is in contrast to other studies[56,95,96]. This is most likely due to the small differences in ventilation rate and humidity between sites, which is a result of enforcement of a Dutch law on laboratory animal welfare.

The average levels of the highest exposed tasks were approximately 10 times higher than the average ambient air levels found in the animal rooms (figure 1 and 2). However, the allergen levels of the ambient air and personal samples were not completely comparable. The ambient air samples measured the total dust fraction, whereas the personal measurements were restricted to inhalable dust, which is a smaller dust fraction[102]. Total dust samplers were used for ambient air sampling in order to collect more dust for detection of the allergens. Additionally, the cascade impactor samples showed that a large proportion of the RUA and MUA were present on particles larger than 9 μm (table 3). Therefore, under similar conditions the total dust samples will contain relatively more allergens than the inhalable dust samples. As a result, the presented differences between the concentrations derived from the personal and ambient air samples would even be larger.

RUA and MUA were found mainly on particles larger than 5.8 μm, which is consistent with findings in other studies[59,61,68,94,103]. These particles are most likely to deposit in the nose and upper airways[104]. This is in agreement with the observation that allergic symptoms of the upper airways, i.e. nose or eye symptoms (rats, 16.8%; mice, 9.0%), are most prominent and are more prevalent than chest tightness (asthma) (rats, 6.1%; mice, 3.2%)[83].

In another paper (Chapter 6)[86] we described a relationship between exposure to RUA

and the prevalence rate of rat allergy. This relationship was more pronounced in 'atopic' workers. However, even at low 'time-multiplied exposure' levels (< 1 hours weekly ng eq/m^3) 9% of the 'atopic' workers, had an allergy to rats. In contrast, among the 'non-atopic' workers, allergy to rats was not present in the lowest exposure group. On the basis of the measurements presented in this paper, this 'time-multiplied exposure' level could be reached by changing rats for about nine minutes per week (figure 2) or being in an average rat room for slightly more than one hour (figure 1). Consequently, large reduction in exposure is necessary for this group of sensitive workers, i.e. 'atopic' workers, to be able to work under normal conditions with a low risk of developing occupational allergy.

This exposure study revealed some important determinants of rat and mouse urinary allergens exposure, which can be used for further study on exposure control in laboratory animal facilities. Previous studies showed that reduction of ambient air allergens levels in animal rooms is possible by using filter top cages[68], increasing the ventilation rate[96], or increasing the relative humidity[95]. These control measures might, however, only partially reduce a worker's exposure. For instance, the use of filter top cages may result in a low exposure when a room is entered for inspection of animals or for tasks that do not disturb the animals. For handling animals or removing the contaminated bedding, tasks which were related to high exposures, these filter tops have to be removed and will hardly reduce the exposure during the performance of these tasks. Therefore, exposure control is probably more effective if measures are taken to prevent allergens getting airborne during the tasks leading to high exposure levels, than taking measures to reduce the ambient air allergen levels.

The presented determinants of RUA and MUA exposure will also be used to estimate exposure levels in our epidemiological study on LAA. The task and site specific allergen exposure levels may be used in combination with detailed information on the time spent the different tasks. However, due to the large influence of site on the personal exposure level, the results can not be generalised to other studies on LAA.

In conclusion, this study showed that number of animals present in the room, use of filter top cages, and use of infrared lights were important determinants of ambient air RUA and MUA levels in Dutch laboratory animal facilities. The RUA and MUA exposure levels were predominantly determined by the task performed and the site in which this was performed.

CHAPTER 4

COMPARISON OF THREE METHODS TO ASSESS AIRBORNE RAT AND MOUSE URINARY ALLERGEN LEVELS

ABSTRACT

Background: Methods to quantify airborne laboratory animal allergens have been developed by various research institutes in order to study exposure-response relationships and identify sources of exposure. However, methods developed may vary on various aspects, e.g. elution, immunoassay or antibody source.

Objective: As part of an European study, methods to measure rat and mouse urinary aeroallergens of three institutes were compared.

Methods: In total 222 (3 * 74) ambient air inhalable dust samples were taken in animal facilities in the Netherlands, UK and Sweden. After elution the extracts were analysed on rat and mouse urinary allergen content.

Results: Rat allergen levels found by the method developed in the UK, which was based on RAST-inhibition assay, were 3000 and 1700 times higher compared to the levels measured by the methods developed in the Netherlands and Sweden, both based on EIA-sandwich assay. The difference between the methods developed in Sweden and the Netherlands was much smaller, 2.2 times. The differences were smaller for the mouse allergen levels, 4.6, 5.9 and 1.6 times, respectively. The methods developed in the Netherlands were the least sensitive and the methods developed in the UK were the least specific. The addition of Tween to the elution buffer and type of antibodies used in the assay (monoclonal/polyclonal), were identified as factors causing the differences between methods developed in Sweden and the Netherlands.

Conclusions: Large differences can be found between the various methods to measure rat and mouse aeroallergens. The use of conversion factors to make data from previously performed allergen measurements comparable or exchangeable is limited and thorough standardisation of methods is preferred.

---

by Albert Hollander, Anne Renström, Susan Gordon, Joost Thissen, Gert Doekes, Per Larsson, Kate Venables, Per Malmberg and Dick Heederik, submitted for publication.

INTRODUCTION

Laboratory animal workers are at risk of developing occupational allergy[12-14,17,18,22,34,83]. The risk of developing laboratory animal allergy (LAA) has been found to be associated with the level of allergen exposure[18,22,86]. In all three studies which showed an exposure-response relationship, the exposure to airborne laboratory animal allergens was quantified using sensitive immunoassays. In other studies[58,59,62,68,93,101] these immunoassays have been used to identify determinants of laboratory animal allergen exposure. Yet, allergen concentrations presented in the studies should be compared with care[64]. The reported allergen levels may differ due to the sampling equipment and extraction methods used. In addition, differences in allergen concentrations may be due to the reference allergens used in the immunoassay, e.g. one 'major' urinary allergen or a pool of urinary allergens, and may also depend on the antibodies used, e.g. antibodies derived from sensitised workers, or polyclonal or monoclonal antibodies raised in immunised animals. Furthermore, the type of immunoassay used, e.g. an inhibition or sandwich immunoassay, may contribute to the differences in allergen levels reported.

As part of the concerted action program 'Epidemiology of occupational allergic asthma and exposure to bio-aerosols' supported by the European Union (contract no. BMH1-CT94-1446), methods to measure rat urinary aeroallergens (RUA)[63,64,100] and mouse urinary aeroallergens (MUA)[65,100,105] in inhalable dust samples were compared. These methods were developed by three European research groups, i.e. the National Heart and Lung Institute, London (NHLI), Wageningen Agricultural University (WAU) and National Institute for Working Life, Solna (NIWL). The methods have previously been used to study exposure-response relationships[22,86] and to identify determinants of exposure[68,93,100,101].

MATERIALS AND METHODS

STUDY DESIGN

The study design is presented in figure 1. In total 222 ambient air inhalable dust samples were taken in a laboratory animal facility in the Netherlands (105 samples), UK (42 samples) and Sweden (75 samples). The samples were taken in rat rooms, mouse rooms and rooms in which rat and mouse cages were cleaned. Each set of parallel samples consisted of three identical inhalable dust samples. The sampling was performed according to the method routinely applied by each institute (table 1). For each parallel set the three sampling heads were randomly placed approximately 15 cm apart of each other on the left, middle or right of the sampling stand. The sampling time was varied between 40 minutes and 20 hours (mean 460 minutes) to provide a

sufficiently wide range of RUA and MUA concentrations. In total 74 sets of parallel filters (74 * 3 filters) and 18 sets of blank filters (18 * 3), i.e. samples without air drawn through the filter, were obtained.

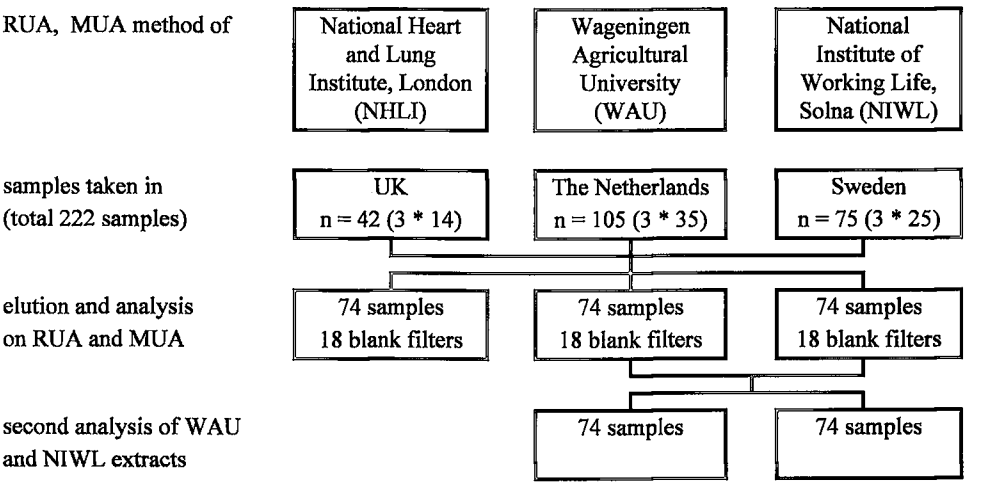


Figure 1. Study design.

Immediately after sampling, each institute received a set of 92 filters. Within three months after sampling each institute eluted and assayed these 92 filters. The elution methods and immunoassays of the three institutes have been described in full detail elsewhere[63-65,100]. Essential features and differences between the methods are described in table 1. All filter extracts were analysed on rat as well as on mouse urinary allergen content. The filters were extracted and analysed in such as way, that blind testing was ensured.

In order to study the influence of elution method and immunoassay separately, the extracts produced and analysed by the WAU (74 extracts) and NIWL (74 extracts) were exchanged and analysed subsequently. At the same time each institute analysed its own extract again in order to account for differences due to the lack of sufficient reproducibility or to more prolonged storage. The extracts of the NHLI were not included in the second analysis, because insufficient sample material remained after the initial analyses.

STATISTICAL ANALYSIS

The detection limit of each method has been determined elsewhere[63-65,100]. These limits are presented in table 1. In this study these limits and the mean sampled volume, 0.92 m^3, were used to calculate the detection limit per m^3. All samples with

Table 1 *Essential features and differences between the methods developed by the three institutes to measure airborne rat and mouse urinary allergen levels*

	institute		
	NHLI[63,65]	WAU[100]	NIWL[64]
sampling method			
inhalable dust sampler	seven-hole	IOM	IOM
PTFE filter	pore size 1.2 mm	pore size 1.0 mm	pore size 1.0 mm
elution method			
buffer	2 ml 0.1 M NH_4HCO_3 + 0.5% Tween 20	2 ml 0.15 M PBS	1 ml 0.15 M PBS + 0.5% Tween 20
method (extracts were all stored at -20°C)	vortexed, centrifuged, and lyophilised. Reconstituted in PBS + 0.3% w/v HSA before assay to get 10 fold concentrated extract	vortexing 2 min, sonicating 2 min, vortexing 5 min, sonicating 2 min and centrifuged	rotation 1 hour, filter was discarded and 1% w/v BSA was added
RUA immunoassay			
immunoassay	radioallergosorbent test (RAST) inhibition	enzyme immunoassay (EIA) sandwich	EIA-sandwich
rat standard preparation (urinary proteins)	from male, post-pubertal Wistar rats	from young/old and male/female Wistar rats	*Rat n* I from 3-4 month male Sprague Dawley rats
antibodies	IgE pool of 8 rat allergic workers	polyclonal antibodies against RUA	monoclonal antibodies against *Rat n* I
detection limit assay	50 ng dry weight/ml	0.075 ng protein/ml	0.10 ng protein/ml
detection limit method	10 ng per filter (10.9 ng/m^3)	0.15 ng per filter (0.16 ng/m^3)	0.10 ng per filter (0.11 ng/m^3)
MUA immunoassay			
immunoassay	RAST-inhibition	EIA-sandwich	EIA-sandwich
mouse standard preparation (urinary proteins)	from male, post-pubertal mice	from young/old and male/female Balb/c mice	*Mus m* I from post pubertal male NMRI mice
antibodies	polyclonal antibodies against MUA	polyclonal antibodies against MUA	polyclonal antibodies against *Mus m* I
detection limit assay	0.5 ng dry weight/ml	0.075 ng protein/ml	0.10 ng protein/ml
detection limit method	4.0 ng per filter (4.3 ng/m^3)	0.15 ng per filter (0.16 ng/m^3)	0.10 ng per filter (0.11 ng/m^3)

undetectable RUA or MUA levels were given two third of the detection limit[106].
All statistical analyses were performed using SAS software (version 6.09). The distributions of the RUA and MUA levels were not clearly normally or log-normally distributed. Therefore, the median levels of these distributions were presented. The level of agreement between methods was described by calculating the geometric mean of the relative difference (ratio) between the RUA and MUA levels detected by the methods in each sample[107]. In addition, a 95% confidence interval (CI) of the relative difference was calculated for each comparison. The correlation coefficient for each comparison was also presented.

RESULTS

SAMPLING STATISTICS

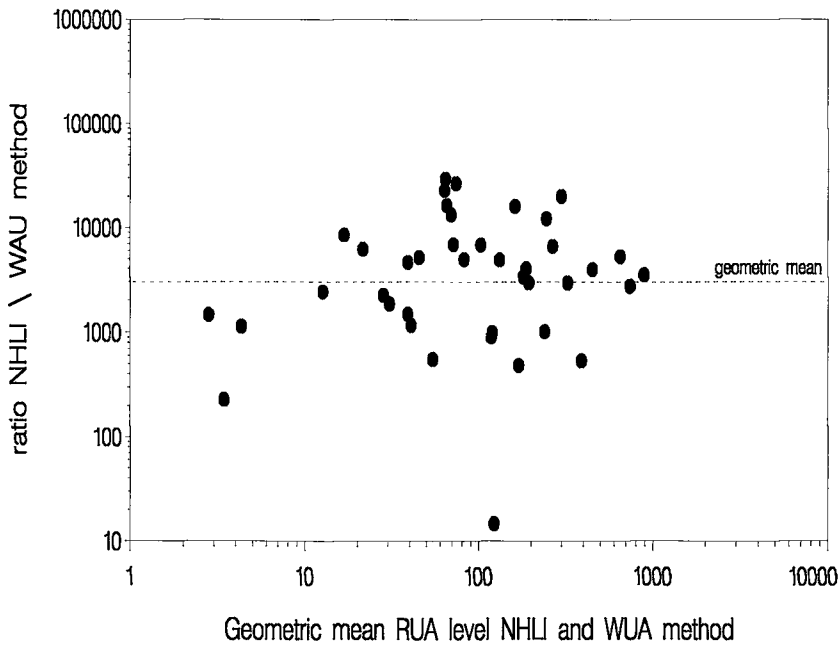
Table 2 shows the descriptive statistics of the RUA and MUA levels found by the methods of the three institutes. Some of the 74 samples were missing due to problems during the elution, or because insufficient extract remained after the RUA analyses (MUA analyses of the NHLI). The RUA concentrations found by the method of the NHLI were several orders of magnitude higher compared to the concentrations measured by the other methods (table 2). These differences were visible in the samples taken in all three countries. The concentrations found by the MUA method of the NHLI were also higher compared to the concentrations measured by the other two methods. However, these differences were much less compared to the differences found by the RUA methods.

Table 2. *Median and range of RUA and MUA levels (ng/m^3) found in ambient air dust samples taken in de facilities of the three participating countries. The levels are stratified by the institute analysing the filters.*

	filters taken in								
	UK			The Netherlands			Sweden		
method	N	median (ng/m^3)	range	N	median (ng/m^3)	range	N	median (ng/m^3)	range
RUA									
NHLI	13	11000	172 - 52900	35	3730	< 10.9 - 47200	25	775	< 10.9 - 21700
WAU	13	0.37	< 0.16 - 15.0	35	0.86	< 0.16 - 31.9	25	< 0.16	< 0.16 - 3.6
NIWL	14	1.95	< 0.11 - 11.8	35	2.0	< 0.11 - 43.4	25	0.71	< 0.11 - 11.6
MUA									
NHLI	10	6.7	2.1 - 163	21	10.4	2.1 - 4610	20	8.8	2.1 - 82.5
WAU	13	< 0.16	< 0.16 - 32.6	34	1.1	< 0.16 - 1560	25	< 0.16	< 0.16 - 3.0
NIWL	14	0.24	< 0.11 - 71.5	35	2.8	0.13 - 446	25	0.36	< 0.11 - 6.1

RUA METHODS

Of the samples presented in table 2, samples with RUA concentrations above the detection limit were used to quantitatively compare the methods of the three institutes. The relative differences (ratios) of the values measured by the various RUA methods are presented in figure 2. The number of samples for each comparison can vary, because the ratios were only calculated when methods of both institute gave detectable values. A close agreement between methods would have resulted in a distribution of the ratios around one. The concentrations found by the RUA method of the NHLI were 3000 (n = 40, 95% CI 1900 - 4900; $r^2 = 0.31$) and 1700 (n = 56, 95% CI 1200 - 2500; $r^2 = 0.35$) times higher compared to the concentrations measured by the RUA methods of the WAU and NIWL, respectively. The geometric mean of the relative difference between RUA concentrations found by the methods of the NIWL and WAU was much smaller, 2.2 times (n = 38, 95% CI 1.6 - 3.1; $r^2 = 0.45$).



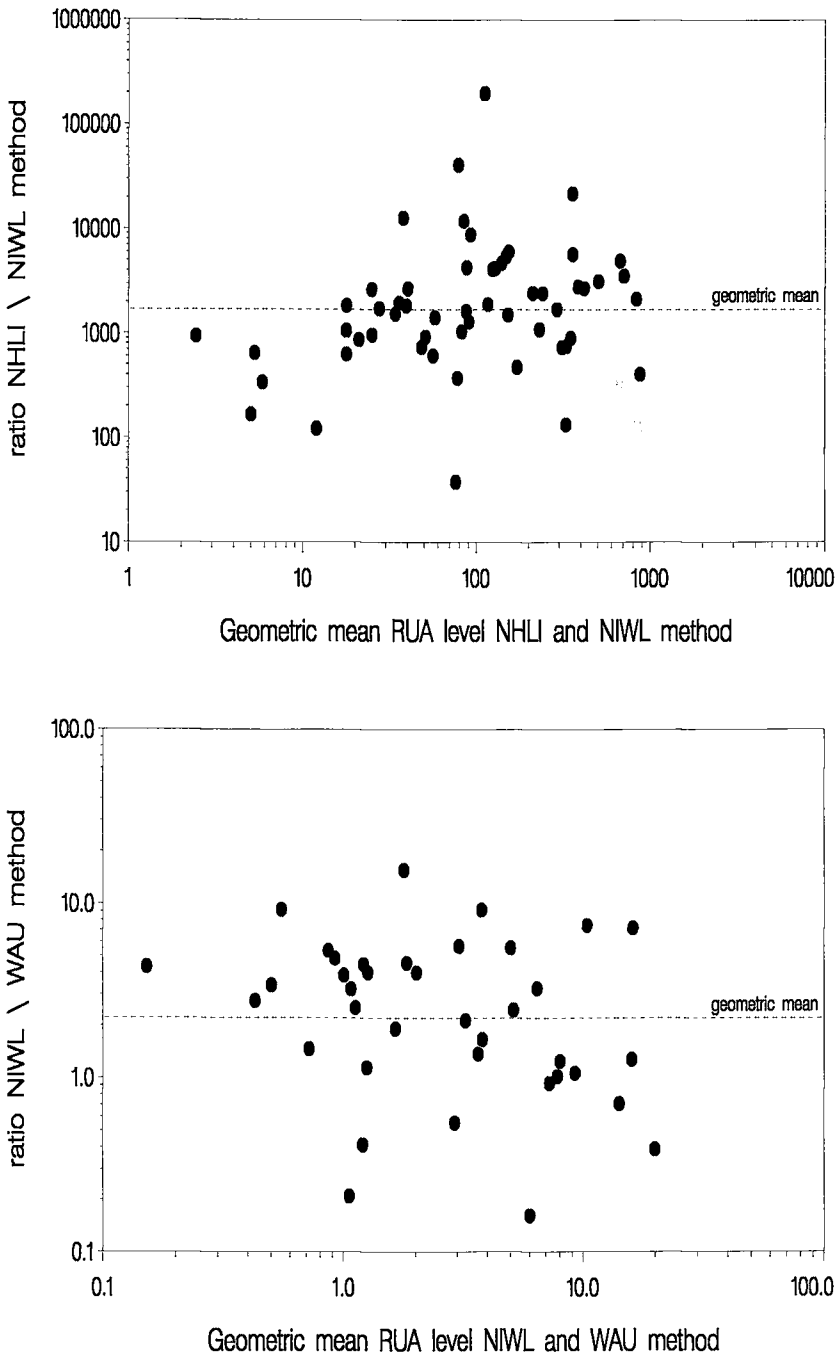
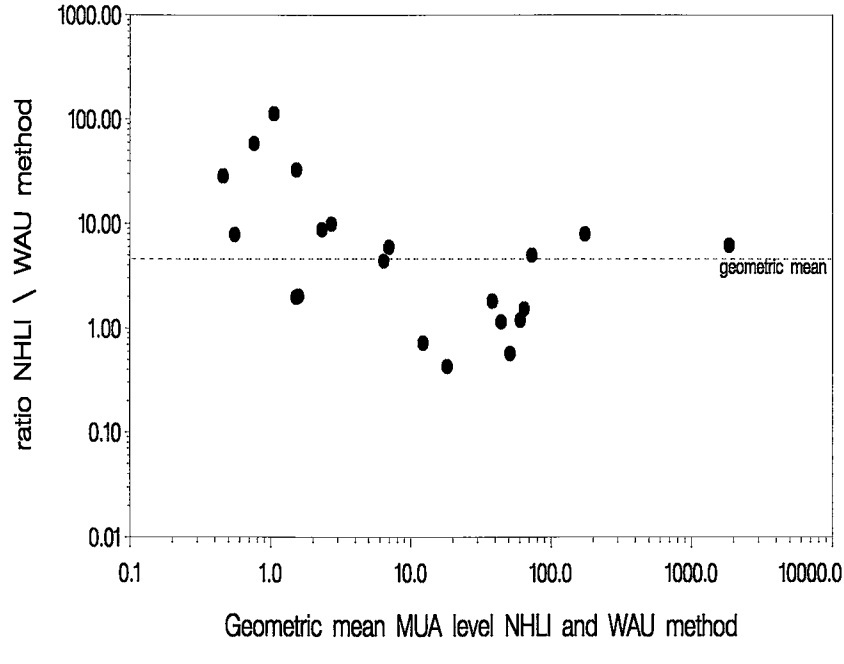


Figure 2. *Relative difference (ratio) against the geometric mean for RUA levels measured by the methods of the institutes: National Heart & Lung Institute (NHLI), Wageningen Agricultural University (WAU) and National Institute for Working Life (NIWL).*

MUA METHODS

Similar to the RUA methods, only samples with MUA concentrations above the detection limit were used to quantitatively compare the methods of the three institutes. The relative differences (ratios) of the values measured by the various MUA methods are presented in figure 3. The MUA concentrations found by the method of the NHLI were also significantly higher compared to the concentrations measured by the MUA methods of the WAU and NIWL, 4.6 times (n = 21, 95% CI 2.3 - 9.1; $r^2 = 0.68$) and 5.9 times (n = 34, 95% CI 3.5 - 9.8; $r^2 = 0.64$), respectively. However, these differences were much smaller compared to the differences found by the RUA methods. Furthermore, the MUA method of the NHLI gave relatively higher levels at low MUA levels and showed the opposite at high MUA levels when compared with the other two MUA methods (figure 3). These differences suggest that the relationship between the MUA method of the NHLI and the MUA methods of the other two institutes is concentration dependent. Again, the geometric mean of the relative difference between the MUA concentrations found by the methods of the NIWL and WAU was smaller, 1.6 times (n = 32, 95% CI 1.0 - 2.5; $r^2 = 0.80$).



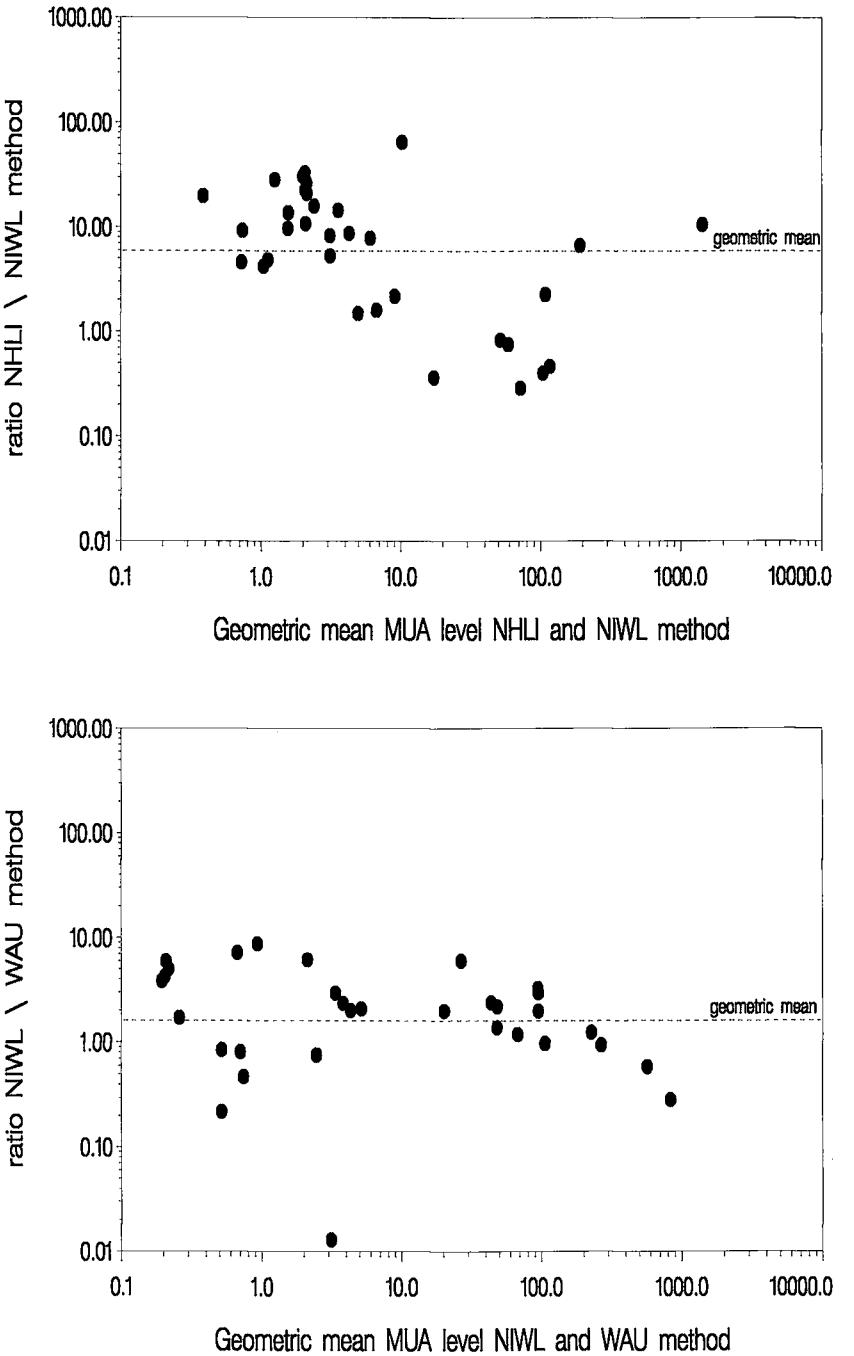


Figure 3. *Relative difference (ratio) against the geometric mean for MUA levels measured by the methods of the institutes: National Heart & Lung Institute (NHLI), Wageningen Agricultural University (WAU) and National Institute for Working Life (NIWL).*

RUA AND MUA LEVELS IN RAT AND MOUSE ROOMS

The filters were taken in rat and mouse rooms. The 'sensitivity' and 'specificity' of the methods were calculated on the basis of the number of samples above and below the detection limit of the methods (table 3). The RUA method of the NHLI and NIWL were both very 'sensitive'. In 97% of the samples taken in rat rooms, these two methods were able to detect RUA, whereas the method of the WAU was able to detect RUA in only 76% of the samples. In contrast, the method of the WAU was more 'specific', i.e. 91% of the samples in mouse rooms had no detectable RUA levels. The RUA method of the NIWL was also relatively 'specific' (77%), but the RUA method of the NHLI detected RUA in most samples taken in mouse rooms ('specificity' = 9.5%). The lower 'specificity' of the RUA method of the NHLI was also shown by the analyses of the blank filters (table 3). The method of the NHLI detected RUA levels above the detection limit in 44% of the blank filters, range < 10.9 to 1290 ng/m^3. The methods of the WAU and NIWL only detected RUA levels in 6% (range < 0.16 to 0.42 ng/m^3) and 17% (range < 0.11 to 0.72 ng/m^3) of the blank filters, respectively. The MUA methods gave similar results (table 3).

Table 3. *RUA and MUA levels (ng/m^3) found on filters taken in rat rooms, mouse rooms, and on the blank filters by the methods of the three institutes.*

	rat rooms			mouse rooms					blank filters		
method	N	N_d*	median (ng/m^3)	N	N_d*	median (ng/m^3)	sensitivity† (%)	specificity‡ (%)	N	N_d*	range (ng/m^3)
RUA											
NHLI	38	1	6960	21	2	330	97	9.5	18	10	< 10.9 - 1290
WAU	38	9	0.62	22	20	< 0.16	76	91	17	16	< 0.16 - 0.42
NIWL	38	1	2.1	22	17	< 0.11	97	77	18	15	< 0.11 - 0.72
MUA											
NHLI	29	11	2.9	13	1	17.0	92	38	10	7	< 4.3 - 5.6
WAU	38	27	< 0.16	21	7	3.0	67	71	16	15	< 0.16 - 0.43
NIWL	38	12	0.29	22	3	17.3	86	32	18	17	< 0.11 - 0.18

* N_d, number of samples below the detection limit

† $(N - N_d)/N * 100\%$ for the RUA values in rat rooms or MUA values in mouse rooms

‡ $N_d/N * 100\%$ for the RUA values in mouse rooms or MUA values in rat rooms

SECOND ANALYSIS

In order to study the influence of elution method and immunoassay separately, the extracts produced and analysed by the WAU and NIWL were exchanged and analysed subsequently (figure 1). Between the initial and second analysis there was a period of

approximately 9 months. In order to account for differences due to the lack of sufficient reproducibility or to more prolonged storage, each institute performed a second analysis on its own extract simultaneously. Again, only samples with detectable allergen level were used in the analyses. The second analysis by RUA and MUA method of the WAU showed allergen levels, which were on average 63% (n = 35, 95% CI 53% - 71%; r^2 initial and second analysis = 0.89) and 38%(n = 28, 95% CI 30% - 50%; r^2 initial and second analysis = 0.96) of the levels measured in the initial analysis, respectively. These differences were much less for the methods of the NIWL, RUA levels of second analysis were on average 77% of the initial levels (n = 50, 95% CI 71% - 91%; r^2 initial and second analysis = 0.92) and MUA levels of second analysis were on average 109% of the initial levels (n = 57, 95% CI 100% - 120%; r^2 initial and second analysis = 0.97).

Table 4. *Geometric mean and 95% CI of the relative difference (ratio NIWL/WAU) of RUA and MUA levels due to the method of elution.*

parallel extract of	analysed by immunoassay of	N	geometric mean ratio NIWL/WAU due to elution method	95% CI	r^2
RUA					
WAU and NIWL	WAU	37	11.2	7.4 - 17.0	0.48
WAU and NIWL	NIWL	9	6.1	2.0 - 19.1	0.01
MUA					
WAU and NIWL	WAU	49	4.8	3.4 - 6.8	0.82
WAU and NIWL	NIWL	18	6.3	2.8 - 13.9	0.63

DIFFERENCES DUE TO ELUTION METHOD

Firstly, the data of the second analysis were used to study the influence of elution method. Therefore, allergen levels in the extracts of the WAU and NIWL measured by the RUA and MUA immunoassay of the NIWL were compared (table 4). The RUA immunoassay of the NIWL was only able to detect RUA in 9 parallel extracts. Comparison of the RUA levels in these 9 parallel extracts showed that the elution method of the NIWL gave 6.1 times (95% CI 2.0 - 19.1) higher RUA levels than the elution method of the WAU. However, due to detection problems the correlation between the RUA levels in both extract was very low (r^2 = 0.01, table 4). A similar comparison was performed with allergen levels of the WAU and NIWL extracts measured by the RUA and MUA immunoassay of the WAU (table 4). Again, a higher RUA level due to elution method of the NIWL was found, 11.2 times (37 parallel extracts had detectable RUA levels, 95% CI 6.8 - 14.6). When the results of the RUA

immunoassay of both institutes were combined, the elution method of the NIWL gave 10 times (n = 46, 95% CI 6.8 - 14.6) higher RUA levels compared to the elution method of the WAU. Similar results were found by the MUA immunoassays (table 4). When the MUA results were combined, the elution method of the NIWL gave 5.1 times (n = 67, 95% CI 3.7 - 7.1) higher MUA levels compared to the elution method of the WAU.

DIFFERENCES DUE TO IMMUNOASSAY

Secondly, the data of the second analysis were used to study the influence of immunoassay. When the extracts of the NIWL were analysed, the RUA levels found by the immunoassay of the NIWL were 4.3 times (n = 50, 95% CI 3.8 - 5.0) lower compared to RUA levels detected by the immunoassay of the WAU (table 5). A similar result was found when the extracts of the WAU were analysed, 2.6 times (n = 9, 95% CI 0.83 - 7.7) lower RUA levels by the NIWL immunoassay. When these results were combined, the immunoassay of the NIWL gave 4.1 times (n = 59, 95% CI 3.4 - 4.9) lower RUA levels compared to the immunoassay of the WAU. The differences between the two MUA immunoassays was not consistent (table 5). When the MUA results of the extracts of the WAU and the NIWL were combined, the MUA immunoassays of both institutes gave similar results (n = 68, ratio NIWL/WUA immunoassay = 1.1, 95% CI 0.91 - 1.3).

Table 5. *Geometric mean and 95% CI of the relative difference (ratio NIWL/WAU) of RUA and MUA levels due to the immunoassay.*

parallel extract of	analysed by immunoassay of	N	geometric mean ratio NIWL/WAU due to the immunoassay	95% CI	r^2
RUA					
NIWL	WAU and NIWL	50	0.23	0.20 - 0.26	0.87
WAU	WAU and NIWL	9	0.38	0.13 - 1.2	0.53
MUA					
NIWL	WAU and NIWL	53	1.4	1.1 - 1.6	0.96
WAU	WAU and NIWL	15	0.59	0.40 - 0.83	0.94

DISCUSSION

Although LAA is highly prevalent among laboratory animal workers and several studies[18,22,86] have found an association between the level of allergen exposure and the prevalence rate of LAA, there is no standard method available for measuring laboratory animal allergen levels in airborne dust samples. This study shows that large

differences in allergen level can be found, which are due to the method used. Of the methods investigated in the study, especially the methods measuring RUA, showed large differences in allergen level.

A thorough assessment of aeroallergen exposure in laboratory animal facilities requires a sensitive aeroallergen assay[58,59,62,68,93,101]. The methods for measuring RUA and MUA were all highly sensitive. However, the Dutch methods showed to be the least sensitive, i.e. 24% of the RUA and 33% of the MUA samples had undetectable allergen levels in samples taken in rat and mouse rooms, respectively. However, the Dutch methods showed to be more specific. In 91% of the samples taken in mouse rooms no RUA could be detected. Additionally, the RUA method of the WAU detected in only one blank filter rat allergens. In contrast, the RUA method of the NHLI detected RUA in more than 90% of the samples taken in mouse rooms and in 44% of the blank filters. The RUA method of the NIWL was sensitive as well as specific.

Differences between RUA and MUA levels detected by the various methods can be due to differences in dust sampling method, elution method or immunoassay (table 1). All three institutes used inhalable dust samplers, i.e. the NIWL and WAU used the IOM sampling head and the NHLI the Seven-hole sampling head. Two studies[108,109] showed that the IOM sampling head measured 1 to 1.2 times higher dust levels compared to the Seven-hole sampling head. Therefore, the difference in sampling head used could not account for the large differences between the RUA and MUA levels detected by the various methods.

The elution method can affect the RUA and MUA levels found in air dust samples. The elution method of the NIWL gave approximately 10 and 5 times higher RUA and MUA levels than the elution method of the WAU, respectively. The major differences between the methods were agitation technique and the addition of Tween (table 1). One study[81] has found a small effect of the agitation methods used. Sonication plus vortexing showed a 13% higher yield than gentle shaking[81]. However, this study used proteins derived from potatoes[81], which might explain why type of elution technique did not significantly affect RUA yields recovered[66]. In addition, this study[66] has reported a the three-fold increase in RUA yield when adding 0.5% Tween 20 to the elution buffer. In our study the differences in elution between the WAU and NIWL methods is, therefore, probably due to the addition of Tween to the elution buffer of the NIWL.

The immunoassay used has an effect on the allergen level found in air dust samples. In our study the RUA immunoassay of the WAU gave approximately 4 times higher values than the NIWL immunoassay. In contrast, the MUA immunoassays showed no

significant difference in allergen levels between the WAU and NIWL assay. The differences between the RUA levels derived from the WAU and NIWL immunoassays may be due to (1) the reference preparations and (2) type of antibodies used (table 1). The differences could not be explained by type of immunoassay used, because the both institutes used a sandwich immunoassay. However, type of immunoassay may partly explain the differences between the methods of the NHLI, which are based on inhibition assays, and the methods of the two other institutes.

Firstly, the differences between the allergen levels derived from the WAU and NIWL immunoassays may be due to the reference preparations used. In the immunoassays, urinary proteins of rats and mice were used as reference preparations. Urine comprises most important allergens found in airborne dust in laboratory animal facilities[46,110]. The different standards showed extensive allergenic similarity in the different immunoassays, despite the different strains of animals which were used (paper in preparation). Therefore, differences in strain of animal used for the reference preparations will probably not play an important role in the differences found between the assays[46,48].

Secondly, the differences between the allergen levels derived from the WAU and NIWL immunoassays may be due to type of antibodies used. A major difference between the assays is the type of antibodies used. This was clearly visible when the RUA and MUA methods were compared. Both the WAU and NIWL used for the MUA assay polyclonal antibodies and this resulted in only minor differences between assays. Similarly, the MUA method of the NHLI also used polyclonal antibodies and the small difference between this method and the method of the WAU and NIWL, 4.6 and 5.9 times higher, respectively, may be due to the inhibition assay used. In contrast, for the RUA immunoassay different sources of antibodies were used. The polyclonal rabbit antibodies used in the WAU immunoassay reacted with all allergens present in rat urine[100]. The immunoassay of the NIWL used a monoclonal antibodies against *Rat n* I and thus did not measure the other allergens which were present in the air samples. Together with the use of purified *Rat n* I as reference, this resulted in 4 times lower RUA levels compared to the levels derived with the immunoassay of the WAU. More detailed information on the differences between the immunoassays of the three institutes will be described elsewhere (in preparation).

The comparison of assays performed in this studies is the first step towards standardisation of methods to measure RUA and MUA. In general, the differences between the methods were systematic and conversion factors may be used to compare or exchange data of already performed exposure measurements. However, there are several limitations of the use of these conversion factors. Firstly, the MUA assay of

the NHLI showed a deviation from a linear relationship when compared with the methods of the other institutes (figure 3). This is probably due to the unreliability of the method of the NHLI at low MUA concentrations, which might also explain the high number of blank filters with detectable MUA levels. Secondly, the time between elution and analysis of a sample might be important. The methods of the MUA showed a large decrease in allergens level after a storage period of the extract of 9 months at - 20°C. This decrease was not present in the extracts of the NIWL. This was probably due to the addition of BSA to the elution buffer. Finally, additional limitations of the use of conversion factors are differences in sampling strategy, and definitions of job titles and tasks.

In conclusion, large differences can be found between the various methods to measure aeroallergens. Possible causes are the addition of Tween to the elution buffer, type of immunoassay (inhibition versus sandwich), standard preparation (one urinary protein versus multiple proteins) and type of antibodies (IgE antibodies derived from sensitised workers, or polyclonal or monoclonal antibodies raised in immunised animals). These items should be included in further standardisation of methods, which is a necessity for future studies on LAA and possible standard setting.

CHAPTER 5

CAT AND DOG ALLERGY AND TOTAL IGE AS RISK FACTORS OF LABORATORY ANIMAL ALLERGY

ABSTRACT

Background: Laboratory animal workers are at high risk of developing occupational allergy. In many cases the severity of symptoms of allergy makes further work with laboratory animals impossible.

Objective: This study was designed to estimate prevalence rates of sensitisation and symptoms of allergy in a population of laboratory animal workers and to determine the association between various host factors and these prevalence rates.

Methods: A cross-sectional survey was undertaken in 540 workers at eight facilities in the Netherlands. All participants completed a questionnaire and underwent skin prick testing with common and occupational allergens. In addition, total and specific IgE measures were obtained.

Results: Prevalence rates of allergy symptoms caused by working with rats and mice were 19% and 10%, respectively. Symptoms, especially chest tightness, were strongly related to sensitisation. Rat and mouse allergy, defined as symptoms of allergy accompanied by specific atopic sensitisation, were highly associated with elevated total IgE, reported adverse reactions, and positive skin prick test responses to common allergens. This relationship could be explained by a response to cat or dog allergens.

Conclusions: Allergy to cats or dogs seemed to be an important risk factor for laboratory animal allergy, whereas allergy to pollen or house dust mite, in the absence of cat and dog allergy, appeared to be insignificant. More conclusive evidence about cat and dog allergy preceding laboratory animal allergy can only be provided after analysis of follow-up data.

---

by Albert Hollander, Gert Doekes and Dick Heederik, Journal of Allergy and Clinical Immunology 1996;98:545-554

INTRODUCTION

Laboratory animal workers are at risk of developing work-related allergic asthma, rhinitis, conjunctivitis, and/or urticaria. Cross-sectional epidemiological studies have reported prevalence rates of these allergic conditions, also described as Laboratory Animal Allergy (LAA), ranging from 11% to 44%[8-22]. The most commonly reported symptoms are rhinitis and conjunctivitis. Asthmatic symptoms, prevalence rates ranging from 4% to 12%, are mostly accompanied by other respiratory symptoms and are considered end-stage LAA[12,17,20,22]. In many cases these symptoms are severe and make direct or even indirect contact with laboratory animals impossible[9,14].

To estimate the risk of LAA and identify its determinants, a follow-up study among approximately 600 laboratory animal workers is currently in progress in the Netherlands. This article covers three study objectives of the 'base-line' part of this study. First, the prevalence rates of the various different symptoms of allergy attributable to rats and mice were assessed and compared with prevalence rates found in studies in other countries. The majority of studies on LAA did not distinguish between species of animals causing the symptoms, despite possible differences in exposure and potency of allergens of different species. Therefore, the prevalence rates of symptoms of allergy to rats and mice, the species most often used, have been analysed separately in this study.

Second, we studied the associations of reported allergic respiratory symptoms with allergen specific atopic sensitisation, measured by skin prick or specific IgE test. High correlations between symptoms and sensitisation have been reported previously[10-13,17,19,21,22], suggesting LAA to be a 'classical' example of symptomatic type I allergy. However, not all symptoms seemed to be IgE mediated[40] because some workers reported symptoms without detectable specific IgE or a positive skin prick test (SPT) response, which can be of importance in epidemiological studies on LAA.

Finally, the association with host factors, such as gender, smoking and atopy, was quantified. Little is known about gender and smoking as risk factors of LAA. Some studies[22,71] have demonstrated sensitisation to laboratory animals to be more common in smokers. Other studies[10,12,15] have demonstrated no association between smoking and LAA. The effect of gender on the presence LAA has only been studied once[14], and no association could be found. Unlike gender and smoking, atopy has been found to be a strong determinant of LAA[8,11,12,14,17,19,21,34,35,69-71]. However, in these studies different definitions of atopy were used. The strength of the association between atopy and LAA seemed dependent on the definition used[19,21,34,69,70]. We have therefore distinguished various definitions of atopy, based on the basis of total serum IgE level, a self-reported history of symptoms of allergy to common allergens, or a positive SPT

response to common allergens, and quantified the associations of these various definitions of atopy with the prevalence rate of LAA.

MATERIALS AND METHODS

STUDY POPULATION

Employees from laboratory animal facilities of four universities, two research institutes, and a pharmaceutical company and students of a laboratory school participated in the study. All subjects working with small laboratory animals or having contact with material from these animals were invited to participate. Of approximately 750 eligible subjects 579 (77%) participated. Questionnaires were completed by 577 subjects (99.6%), 577 (99.6%) provided blood samples and 542 subjects (94%) were skin prick tested. A completed questionnaire and SPT results were available for 540 participants, and this group was used for the analyses presented in this article. The baseline survey was carried out between June 1992 and December 1993.

QUESTIONNAIRE

A self-administered questionnaire was distributed before the health survey. It was based on a Dutch version of an internationally accepted respiratory questionnaire[78], which has been used previously in other studies on occupational asthma in the Netherlands[29,79]. In addition, questions were asked about personal history of symptoms of allergy to common allergens, history of LAA and hyperresponsiveness. In this paper the responses to the following questions were used:

Personal history of symptoms of allergy to common allergens: 'Are you (or have you been) allergic to one or more agents?' If response was positive, questions were asked on type of symptoms (chest tightness (asthma), runny or sneezing nose, runny or itchy eyes, and itchy or red skin) and putative agents causing the symptoms (house dust, pollen and/or domestic animals).

History of symptoms of allergy to rats or mice: 'Do you have symptoms of allergy during working hours, after contact with certain agents at work?' If response was positive, questions were asked on type of symptoms (chest tightness (asthma), runny or sneezing nose, runny or itchy eyes, and itchy or red skin) and animal species causing the symptoms.

Reported hyperresponsiveness: 'Did you ever have problems with breathing in one of the following situations: when going from warm to cold surroundings, from cold to warm surroundings, during foggy weather or during cold weather?'

Other questions included in the questionnaire regarded smoking history and intensity of contact with laboratory and domestic animals.

SKIN PRICK TESTING

Five common allergens (mixture of two house dust mites *D. pteronyssinus and D. farinae*, SQ 510; mixture of timothy, rye, foxtail, orchard and meadow grass pollen, SQ 293; mixture of birch, hazel and alder pollen, SQ 197; cat fur SQ 555; dog fur SQ 553, all from ALK Benelux, Houten, The Netherlands), four occupational allergens (rat urine 15.79; rat fur 15.09; mouse urine 15.78; mouse fur 15.08, all from ALK Benelux) and positive (histamine 10 mg/ml, in duplicate) and negative controls (phosphate-buffered saline, PBS) were used for the SPT. The concentrations of the common and occupational allergens were 10000 BU/ml and 5 mg/ml, respectively.

For this study rat and mouse urine allergen preparations were produced by ALK Benelux. Urine from Wistar rats and BALB/c mice was collected by use of metabolic cages. Male and female (3:1) pubertal and adult animals (1:1) were used to control for sex and puberty effects on protein content and composition of urine[49]. Proteins in the pooled rat and pooled mouse urine were isolated through extensive dialysis against phosphate-buffered saline and distilled water, and concentrated by lyophilisation. The two freeze-dried urinary allergen preparations (rat 15.79, mouse 15.78) were also used in the immunoblotting experiments and in studies on aeroallergen exposure.

SPTs were performed by using uncoated Phazet needles with 1 mm tip (ALK Benelux). The Phazet needle was pressed at 90 degrees to the skin surface through a drop of the test solution[111]. All SPTs were performed by two skilled technicians. The tests were performed on the volar aspect of the forearm and read after 15 minutes. Weals were traced and transferred to a registration sheet by transparent tape. The mean of the longest and midpoint orthogonal diameters was determined using a drawing board connected to a computer. A weal diameter of 3 mm or more for the common allergens, after subtraction of any response to the negative control, was regarded as a positive response. Similar testing with the five common allergens and rat fur allergens was performed in a control group of 169 bakery workers[29]. In this control group all SPT responses to rat fur were smaller than 4 mm. Therefore, a weal diameter of 4 mm or more for the occupational allergens was regarded as a positive response, after subtraction of any response to the negative control.

The mean weal diameter of the positive controls was 6.6 mm (standard deviation 1.5). The coefficient of variation based on the diameters of both positive controls was 10.6%.

IGE ANTIBODIES

Sera were stored at -20°C until IgE analysis was performed. Blood samples of two workers were missing (n = 538). Specific IgE antibodies to rat urinary allergens

(RUAs) and mouse urinary allergens (MUAs) were measured by immunoassay (AlaSTAT; DPC, Apeldoorn, the Netherlands)[112]. Sera of class 2 or higher (≥ 0.7 kU/l) were considered positive. Total IgE antibodies were measured with a sandwich enzyme immunoassay[113]. Briefly, mouse monoclonal anti-IgE was coated in microwells. Sera were added in four dilutions and incubated for 2 hours at 37°C. Bound IgE was measured after incubation with peroxidase-labelled mouse monoclonal anti-IgE for 1 hour at 37°C, followed by a 30-minute incubation with o-phenylenediamine at 20°C in the dark. The reaction was terminated by the addition of HCl, and the optical density was read at 492 nm. Each microtiter plate included a serially diluted reference sample (Kabi-Pharmacia, 10-9123-01). Elevated total serum IgE was defined as a level above 100 kU/l.

HOST FACTORS RELATED TO ATOPY

Atopy was defined in various ways on the basis of the following parameters: (1) history of symptoms of allergy to common allergens reported in the questionnaire, (2) SPT response to the five common allergens (grass pollen, tree pollen, house dust mite, cat, and dog), (3) total serum IgE.

STATISTICAL ANALYSIS

All statistical analyses were performed using SAS software (version 6.09; SAS Institute, Cary, N.C.). Crude prevalence rates were compared using the χ^2 (or Fisher's exact) test. For testing differences between means, Student's *t* test was used. The effect of host factors on the prevalence rate of LAA was analysed by multiple regression techniques. Prevalence rate ratios (PRRs) were calculated by means of a proportional hazards model (Cox's regression with SAS procedure 'PHREG')[114,115]. Statistical significance was defined as p value of less than 0.05 (two tailed).

RESULTS

PREVALENCE RATE OF SYMPTOMS OF ALLERGY

Allergy symptoms (chest tightness (asthma), eye, nose or skin) attributed to working with laboratory animals were highly prevalent among the laboratory animal workers. Most symptoms of allergy were reported by individuals working with rats. Among the 458 workers who had ever worked with rats, 86 (18.8%) reported at least one rat related allergic symptom. Among individuals who had ever worked with mice (n = 377), at least one mouse related allergic symptom was reported by 10.1% of the workers. Nose or eye symptoms were reported most frequently (figure 1: rats, 16.8%; mice, 9.0%), followed by skin symptoms (rats, 10.7%; mice, 4.2%) and chest tightness

(asthma) (rats, 6.1%; mice, 3.2%). Of all persons with symptoms of allergy caused by working with rats, 52 (60.5%) had more than one symptom. For persons working with mice, this figure was 44.7%. As shown in figure 1, chest tightness (asthma) was rarely present in absence of nose or eye symptoms.

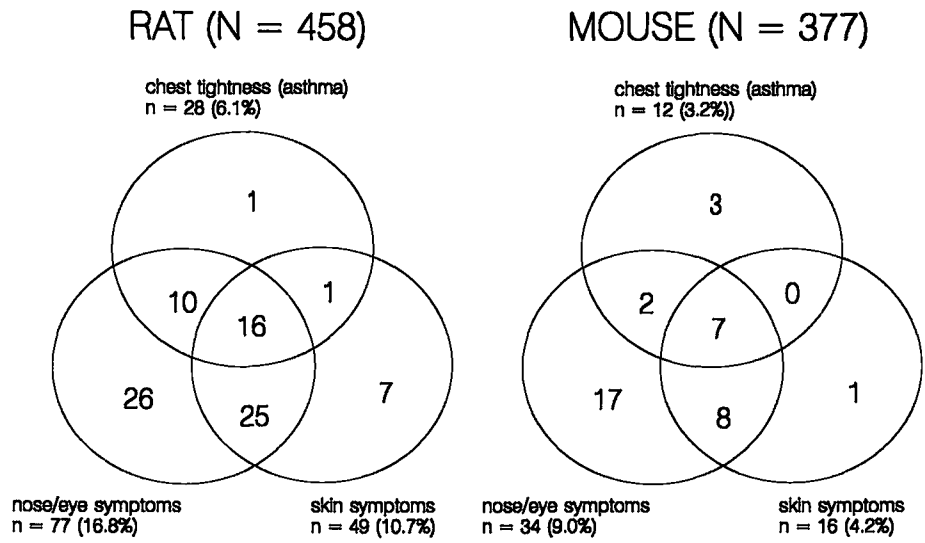


Figure 1. *Prevalence rates of reported symptoms of allergy caused by working with rats and mice.*

Three hundred and forty-two individuals worked with both mice and rats. Of these workers, 26 (7.6%) reported symptoms caused by working with rats, 10 (2.9%) reported symptoms caused by working with mice and 23 (6.7%) reported symptoms of allergy in response to both species.

SYMPTOMS VERSUS SENSITISATION

The prevalence rates of symptoms of allergy and specific sensitisation were nearly identical. Eighty-two subjects working with rats (17.9%) and 37 subjects working with mice (9.8%) had a positive SPT response to urinary or fur allergens, respectively. When sensitisation was measured by specific serum IgE to RUAs or MUAs, the prevalence rates were lower, 11.0% and 6.1%, respectively. Almost all workers with specific serum IgE to RUAs or MUAs had a positive SPT response. When a positive SPT response and/or the presence of specific serum IgE were used as measure of sensitisation to RUAs or MUAs, the prevalence rates increased only slightly to 18.2% and 10.7%, respectively. Symptoms and sensitisation were highly correlated. Of all

workers with symptoms of allergy caused by working with rats, 59 (69%) were sensitised to rat allergens (SPT and/or serum IgE positive). The correlation between symptoms and sensitisation was more evident when chest tightness (asthma) was present (table 1). Sensitisation was rare among workers without symptoms of allergy caused by working with rats, 6.5%. Symptoms caused by working with mice and sensitisation to mouse allergens were also significantly correlated (table 1). However, this correlation was less pronounced.

Table 1. *Sensitisation rates and characteristics of laboratory animal workers with and without allergy symptoms caused by rats or mice.*

	RAT			MOUSE		
	no symptoms	chest tightness (asthma)	other symptoms	no symptoms	chest tightness (asthma)	other symptoms
n	372	28	58	339	12	26
female (%)	39.0	35.7	25.9	38.9	41.7	30.8
smoking (%)	29.1‡	25.0	27.6	30.2‡	16.7	19.2
years working with animals*	11.4 (9.9)	8.4 (7.4)	8.3 (7.3)|	10.3 (9.9)	9.3 (8.7)	10.3 (7.6)
hyperresponsiveness† (%)	11.9‡	42.9§	27.6§	12.8‡	50.0§	19.2
SPT^+ to urinary/fur allergens (%)	6.5	82.1§	60.3§#	6.8	58.3§	26.9§
IgE^+ to urinary allergens (%)	2.4‡	67.9§	37.9§¶	3.9‡	33.3§	23.1§
sensitisation (SPT^+ or IgE^+) (%)	6.5‡	82.1§	62.1§	7.4‡	58.3§	30.8§

* mean (standard deviation)

† respiratory symptoms when going from warm to cold or cold to warm surroundings, or during foggy or cold weather

‡ denominators were 371 and 338 for smoking and 370, 337 for hyperresponsiveness and specific IgE

§ $p < 0.01$ compared to group with no symptoms

| $p < 0.05$ compared to group with no symptoms

¶ $p < 0.01$ compared to group with chest tightness (asthma)

# $p < 0.05$ compared to group with chest tightness (asthma)

Selection out of the work force is often assumed to occur in populations at risk of LAA[17]. Persons with symptoms had worked for shorter periods in laboratory animal facilities than workers without symptoms (table 1), suggesting the presence of the so-called 'healthy worker effect' in these groups.

The population was divided into four groups on the basis of the presence or absence of symptoms of allergy caused by working with rats or mice and sensitisation to the respective animal, defined as positive SPT responses to urinary or fur allergens, and/or serum IgE positive to urinary allergens (table 2). Among those who worked with rats, subjects in the symptomatic/sensitised group reported hyperresponsiveness

significantly more often and had significantly more positive SPT responses to common allergens than subjects in the non-symptomatic/non-sensitised group hyperresponsiveness. The symptomatic/sensitised group also included significantly more men.

Table 2. *Characteristics of workers grouped by presence of allergy symptoms caused by rat or mouse and atopic sensitisation to the respective animal.*

	RAT				MOUSE			
allergy symptoms*	-	-	+	+	-	-	+	+
sensitisation*	-	+	-	+	-	+	-	+
N	346	24	27	59	312	25	23	15
female (%)	39.6	25.0	37.0	25.4‡	39.4	28.0	34.8	33.3
smoking (%)	29.9|	16.7	26.0	27.1	30.6|	24.0	21.7	13.3
reported hyperresponsiveness (%)	11.6|	12.5	37.0†	30.5†	11.3|	28.0‡	39.1†	13.3
positive SPT response (%) to:								
grass pollen	13.9	25.0	22.2	40.7†	16.7	28.0	17.4	33.3
tree pollen	9.5	41.7†	14.8	22.0†	11.2	24.0	17.4	26.7
house dust mite	18.8	37.5‡	40.7†	40.7†	20.5	36.0	56.5†	46.7‡
cat fur	10.7	41.7†	14.8§	44.1†	12.2	36.0†	17.4	33.3‡
dog fur	8.7	54.2†	7.4§	52.5†	10.3	52.0†	21.7§	73.3†

* symptoms of allergy when working with rats or mice; SPT positive to fur or urinary allergens, or IgE positive to urinary allergens

† $p < 0.01$ compared to non-symptomatic/non-sensitised group

‡ $p < 0.05$ compared to non-symptomatic/non-sensitised group

§ $p < 0.01$ compared to symptomatic/sensitised group

| denominators were 345 and 311 for smoking and 344 and 310 for hyperresponsiveness symptoms

The non-symptomatic/sensitised group was comparable with the symptomatic/sensitised group, but included fewer individuals who reported hyperresponsiveness (30.5% versus 12.5%). The mean weal diameter of the SPT response to RUAs in the non-symptomatic group was significantly smaller than in the symptomatic group, 7.6 mm (95% confidence interval (CI) 5.9 - 9.3) versus 10.4 mm (95% CI 9.4 - 11.4), respectively.

Despite the high correlation between reported symptoms and sensitisation not all symptoms seemed to be IgE mediated. Of all subjects who worked with rats, 5.9% reported symptoms in the absence of a positive SPT response to RUAs. This symptomatic/non-sensitised group was largely similar to the non-symptomatic/non-sensitised group, but differed in having significantly higher prevalence rates of self-reported hyperresponsiveness (37.0% versus 11.6%) and of positive SPT responses to

house dust mite allergens (40.7% versus 18.8%). Similar analyses were performed for subjects who worked with mice, but less pronounced results were found (table 2).

Table 3. *Separate analyses of association between gender, smoking, and host factors related to atopy, and prevalence rates of rat and mouse allergy*.*

	RAT				MOUSE			
	n	prevalence rate (%)	PRR	95% CI	n	prevalence rate (%)	PRR	95% CI
gender								
male	288	15.3	1		232	4.3	1	
female	168	8.9	0.58	0.33 - 1.1	143	3.5	0.81	0.28 - 2.4
smoking†								
non-smoker	210	13.3	1		164	4.9	1	
former	115	13.0	0.98	0.52 - 1.8	102	4.9	1.0	0.33 - 3.1
current	130	12.3	0.92	0.50 - 1.7	108	1.9	0.38	0.08 - 1.8
history of symptoms of allergy								
no symptoms	326	8.0	1		275	2.2	1	
symptoms to common allergens	130	25.4	3.2	1.9 - 5.3	100	9.0	4.1	1.5 - 12
symptoms to house dust/ pollen but not to pets	70	15.7	2.0	0.97 - 4.0	56	5.4	2.5	0.61 - 9.8
symptoms to pets	60	36.7	4.6	2.6 - 8.1	44	13.6	6.3	2.0 - 19
positive SPT response to								
none of 5 common allergens	257	5.4	1		214	0.9	1	
at least one allergen	199	22.6	4.2	2.3 - 7.6	161	8.1	8.6	2.0 - 38
grass, tree or mite; negative to cat and dog	84	6.0	1.1	0.40 - 3.0	71	1.4	1.5	0.14 - 17
cat and/or dog fur	115	34.8	6.4	3.5 - 12	90	13.3	14.3	3.2 - 64
total IgE (kU/l)								
< 100	363	7.7	1		305	2.6	1	
≥ 100	93	33.3	4.3	2.6 - 7.2	70	10.0	3.8	1.4 - 11

* symptoms of allergy caused by working with rats or mice *and* sensitisation to the respective animal

† smoking habit of 1 worker was missing

HOST FACTORS

In all further analyses 'rat allergy' and 'mouse allergy' were defined as the presence of symptoms caused by working with rats or mice *and* atopic sensitisation to the respective animal (symptomatic/sensitised group). The analyses were performed by comparing workers who were allergic according to this definition with all other subjects working with the same animal species. The relationships between host factors and rat and mouse allergy are presented in table 3. Rat and mouse allergy were more

prevalent among men than among women, but the difference was not statistically significant. No association was found with smoking (non-smoker, former, or current). All host factors related to atopy were significantly associated with rat and mouse allergy (table 3). For elevated total serum IgE (≥ 100 kU/l) the PRRs of rat and mouse allergy were 4.3 and 3.8, respectively. The prevalence rates of rat and mouse allergy were, respectively, 4.2 and 8.6 times higher when a positive SPT response to at least one of the five common allergens was present. However, the association became stronger when only the SPT responses to cat and dog fur were taken into account (rat allergy PRR, 6.4; mouse allergy PRR, 14.3). Among workers with positive SPT responses to grass pollen, tree pollen or house dust mite allergens but negative responses to cat and dog fur, the prevalence rates of rat and mouse allergy were not increased compared with workers with no response to any common allergen (PRRs of 1.1 and 1.5, respectively). Similar results were found for personal history of symptoms of allergy to common allergens as reported in the questionnaire.

Table 4. *SPT responses to common and rat fur allergens of subjects working with rats compared with the control group of bakery workers*[29].

	working with rats	bakery workers
n	458	169
female (%)	37.1*	11.8
current smoker (%)	28.7*‡	55.0
former smoker (%)	25.2†‡	19.5
positive SPT response (%) to		
one or more common allergens	43.4	38.5
grasses	18.3	20.7
trees	13.1	8.9
house dust mite	23.8	24.3
cat	16.8	16.6
dog	16.6	16.6
rat fur	14.5*	0.0
total IgE ≥ 100 kU/l (%)	20.4‡	21.3

* $p < 0.01$ compared to the bakery workers

† $p < 0.05$ compared to the bakery workers

‡ denominators were 457 for smoking and 456 for total IgE

Cat and dog allergy and elevated total IgE seemed to be important risk factors of rat and mouse allergy. However, in a cross-sectional analysis it is not possible to verify whether a positive SPT response to cat or dog fur or elevated total serum IgE precede

LAA. Therefore, the SPT response to common allergens and the presence of elevated total IgE, have been compared with those of the control group (table 4). SPT responses to all common allergens and the presence of elevated total IgE were similar for control subjects and subjects working with rats.

Table 5. *Multiple regression of the associations between personal history of symptoms of allergy to pets, positive SPT response to cat or dog fur, and elevated total IgE (≥ 100 kU/l) and prevalence rate of rat and mouse allergy*. The associations were controlled for gender and smoking.*

symptoms of allergy to pets SPT response to cat and/or dog total IgE level (≥ 100 kU/l)	RAT				MOUSE			
	n†	prevalence rate (%)	PRR	95% CI	n†	prevalence rate (%)	PRR	95% CI
none present	273	1.8	1.0		233	0.43	1.0	
at least one present	182	29.7	16.0	6.4 - 40	141	9.9	22.2	2.9 - 169
one present	119	21.0	11.2	4.3 - 29	95	6.3	13.9	1.7 - 116
at least two present	63	46.0	24.8	9.6 - 64	46	17.4	39.9	5.0 - 320

* reported symptoms caused by working with rats or mice *and* sensitisation to the respective animal

† smoking habit of 1 worker was missing

Three risks factors for rat and mouse allergy, 'history of symptoms of allergy to pets', 'positive SPT response to cat or dog fur' and 'elevated total IgE', were analysed simultaneously (table 5). When none of the three risk factors was present, the prevalence rates of rat and mouse allergy were very low, 1.8% and 0.43% respectively. Therefore, the PRRs of rat and mouse allergy became respectively 16.0 and 22.2 for workers with at least one risk factor present, controlled for gender and smoking. These PRRs were roughly similar, despite the different prevalence rates of rat and mouse allergy. The PRRs of rat and mouse allergy increased with increasing number of risk factors present, from 11.2 and 13.9, respectively, when one risk factor was present to 24.8 and 39.9, respectively, when at least two risk factors were present.

DISCUSSION

This study is one of the largest on LAA in which symptoms and sensitisation to rats and mice, measured by SPT and specific IgE, have been studied. The overall prevalence rate of symptoms of allergy to rats in our study was 19%, which is roughly similar to prevalence rates found in other studies on rat allergy in the United States (12%[14]), United Kingdom (18%[17], 31%[22]) and Japan (25%[20]). The prevalence rate of mouse-related symptoms was 10% and tended to be lower than described in most other studies (United Kingdom 11%[17]; Japan 26%[20]; Australia 32%[11]). A comparison with other studies should, however, be made with care because of differences in

definitions of allergy to rats and mice and differences in population characteristics and work environment. The 540 workers in our study are a representative selection of approximately 10%[116] of the Dutch laboratory animals workers population. In our study the response rate was 77%. Two important reasons for not participating were a lack of interest in the study and fear of disclosure of animal-related symptoms to the employer. Therefore, the presented prevalence rates could have been slightly higher or lower. Selection out of the work force (healthy worker effect) as suggested in table 1, could also have an effect on the presented prevalence rates. When the population was restricted to workers with less than 4 years of exposure to laboratory animals, the prevalence rate for symptoms of allergy caused by working with rats or mice would only be slightly different, 23.7% and 8.3% respectively. Four years of exposure to laboratory animals was chosen, because most laboratory animal workers were PhD-students with a contract period of 4 years. In this period, selection will be minimal but will still have occurred[22,41].

In exposure-response studies on occupational allergy it is important to make sure that reported symptoms of allergy are indeed provoked by the aeroallergen under study. We therefore assumed that rat allergy and mouse allergy were most likely present if subjects reported symptoms of allergy caused by working with rats or mice and were also sensitised to the corresponding animal (symptomatic/sensitised workers, table 2). Workers with symptoms of allergy (chest tightness (asthma), runny or sneezing nose, runny or itchy eyes, and itchy or red skin) during work after contact with rats or mice, but without being sensitised to rat or mouse allergens (symptomatic/non-sensitised group, table 2) had an elevated prevalence rate of positive SPT responses to house dust mites, and of self-reported hyperresponsiveness compared with workers in the non-symptomatic/non-sensitised group. In a study among pig farmers, the self-reported hyperresponsiveness was compared with bronchial responsiveness to histamine (unpublished data Preller et al.). A mild bronchial responsiveness (i.e. a decrease in FEV_1 of at least 10% at a histamine concentration $\leq$ 16 mg/ml) was correlated with the presence of self-reported hyperresponsiveness (Odds Ratio (OR) = 2.3, $\chi^2 = 4.0$, $p = 0.046$). Thus, the symptoms after contact with rats or mice of workers in the symptomatic/non-sensitised group seemed to be non-IgE mediated but may be the result of a non-specific hyperresponsiveness to animal-derived or other agents, such as dust, disinfectants or ammonia, which are present simultaneously. It is also possible that our cut-off of 4 mm is to stringent. The occupational allergens used for the SPTs were not standardised and seemed more potent than the standardised common allergens, for which 3 mm was used. However, when 3 mm was used as cut-off level for the rat and mouse allergens, only one additional worker with symptoms would be

sensitised, but 12 rat and nine mouse workers without symptoms would be sensitised. Using 3 mm instead of 4 mm will probably lead to more false positive SPT responses and 4 mm is therefore the cut-off level to be used in this study.

We also found a substantial number of sensitised workers without symptoms of allergy (non-symptomatic/sensitised group, table 2). This group should not simply be dismissed as having false-positive results. A recent prospective study[35] showed that among laboratory animal workers without symptoms of LAA, those with sensitisation were much more likely to become symptomatic than those with a negative SPT for rat sensitisation (relative risk of 7.6 after two years of follow-up). Therefore, these workers may be an important group that should be included in a follow-up.

Of the host factors that were studied, smoking was not associated with allergy to rats and mice. Some studies[22,71,117-119] have demonstrated sensitisation to occupational allergens to be more common in smokers. Several other studies[10,12,15,120] have, however, failed to demonstrate a relationship between allergy and smoking. The discrepancy of these results may be partly caused by the cross-sectional design of the studies. It is possible that smoking habits were influenced by the development of symptoms. Therefore the possible association of smoking with LAA requires further study, and smoking habits at the time of first exposure should be known.

LAA was more prevalent in men, but this finding was not statistically significant. Only in one other study on LAA[14] was the effect of gender on the presence of allergy analysed. No significant differences in prevalence rates of LAA between men and women were found. In our study men performed tasks with high levels of aeroallergen exposure more often. The differences in exposure between men and women could account for the higher prevalence rates for men and should be studied in more detail.

The host factors related to atopy were all associated with allergy to rats and mice, which is consistent with findings in other studies[8,10-12,14,17,19,21,22,35]. Interestingly, we found that allergy to cats and dogs was highly associated with rat and mouse allergy. This was also found in two other studies on LAA. In a study among 56 laboratory animal workers[21] a positive SPT response to common allergens was associated with LAA (OR = 9.3, 95% CI 2.4 - 37), but when the response to dog and horse was excluded, the association was much weaker (OR = 3.3, 95% CI 0.7 - 16). In another study[70] five out of ten workers with LAA had positive SPT responses to dog and/or horse, and all workers who were free of symptom had negative SPT responses to these animals. In our study the prevalence rates of rat and mouse allergy in workers without SPT responses to cat and dog fur but a positive response to at least one of the other common allergens (grass pollen, tree pollen, and house dust mite) were comparable with those among workers with no SPT response to any common allergens. Therefore

atopic sensitisation to allergens other than those of pets as risk factor for LAA will be of no additional value and will even have a negative effect because of its high prevalence rate (16%). When a positive SPT response to cat or dog fur was used as risk factor for rat or mouse allergy instead of a positive SPT response any of the common allergens (table 3), the PRRs increased from 4.2 (95% CI 2.3 - 7.6) to 6.2 (95% CI 3.6 - 11), and from 8.1 (95% CI 2.0 - 38) to 12.7 (95% CI 3.6 - 45), respectively.

Elevated total IgE (≥ 100 kU/l) was also strongly associated with rat and mice allergy, which is in agreement with other studies[19,34,70]. When three risks factors (history of symptoms of allergy to pets, positive SPT response to cat or dog fur, and elevated total IgE, were combined in one model, a very strong association with rat and mouse allergy was found (table 5). The high PRRs were partly due to the low prevalence rates among workers with all three risk factors absent. Therefore, cat or dog allergy and elevated total IgE seem to be suitable risk factors to be used in epidemiological studies on LAA.

Due to the cross-sectional nature of our study it was not possible to verify whether the SPT response to pets and an elevated total IgE precede LAA. However, similar prevalence rates of positive SPT responses to cat and dog fur and total IgE were found among bakers (control group, table 4), which makes it more likely that allergy to pets and an elevated total IgE precede LAA. However, more conclusive answers can only be given after analysis of follow-up data.

Despite the higher prevalence rate of symptoms of allergy to rats compared to mice, the PRRs of the host factors (tables 3 and 5) were roughly similar. The host factors seem to have a similar effect on the prevalence rate of rat as well as mouse allergy. The difference in prevalence rate of rat and mouse allergy can possible be explained by other factors like level and intensity of exposure and allergenic potency of both species.

CHAPTER 6

RESPIRATORY ALLERGY TO RATS: EXPOSURE-RESPONSE RELATIONSHIPS IN LABORATORY ANIMAL WORKERS

ABSTRACT

Background: Laboratory animal workers are at high risk of developing occupational allergy. Little is known about the relationship between levels of exposure and the risk of developing laboratory animal allergy.

Objective: This study was designed to quantify the exposure-response relationship for allergy to rats, while controlling for factors like atopy, gender and smoking.

Methods: A cross-sectional study was performed in 540 workers at 8 facilities. All participants completed a questionnaire, underwent skin prick testing with common and occupational allergens, and total IgE as well as occupational allergen-specific IgE antibodies were serologically measured. Personal air dust samples were taken during full-shift periods to estimate the rat urinary aeroallergen exposure levels.

Results: In the whole study population no clear exposure-response relationship was observed. However, in the group of workers with less than 4 years of working experience with laboratory animals the prevalence rate of sensitisation to rat allergens was clearly associated with exposure levels. The exposure-response relationship was steepest for workers with atopy associated risk factors, i.e. self-reported allergy or sensitisation to cats or dogs, or elevated total serum IgE. The prevalence rates of sensitisation to rat allergens for these workers were about 15, 9.5 and 7.3 times higher in the high, medium and low exposure group, respectively, compared to internal reference group.

Conclusions: Our study clearly shows that a relationship between exposure to rat urinary aeroallergens and prevalence rate of allergy to rats exists.

---

by Albert Hollander, Dick Heederik and Gert Doekes, American Journal of Respiratory and Critical Care Medicine 1997;155:562-567

INTRODUCTION

Laboratory animal workers are at high risk of developing work related allergic asthma, rhinitis, conjunctivitis, and/or urticaria. Cross-sectional epidemiological studies have reported prevalence rates of these allergic conditions, also described as Laboratory Animal Allergy (LAA), ranging from 11 to 44%[11-14,17,18,22,72]. However, little is known about the quantitative relationship between the allergen exposure level and the risk of developing LAA. Several studies[11-14,17,18,22] have addressed this issue using various measures of laboratory animal allergen exposure. Crude estimates of exposure, like job title, duration of employment or frequency of contact with animals per year, could not be related to the prevalence rate of LAA symptoms[11,12,17,18]. Four studies[12-14,18] used more quantitative measures of exposure, i.e. hours per day or week working with animals. In only one study LAA symptoms were significantly more prevalent in workers who worked more frequently with animals[18]. However, this finding has not been confirmed in the prospective part of the same study.

Quantitative exposure assessment to aeroallergens by using immunoassays has been applied in two studies[18,22]. In one study three exposure groups were distinguished, based on a large number of full-shift personal air measurements[22]. Allergic skin symptoms were significantly more present at medium and high exposure levels of Rat Urinary Aeroallergens (RUA) compared with the category with low exposure levels. No relationship was observed for chest, eye, and nasal symptoms. In the other study[18] the estimated task-specific rat antigen concentration was multiplied by the duration of performed tasks. This measure of exposure was positively associated with the prevalence rate of LAA symptoms. Results of both studies indicate the importance of measuring personal aeroallergen exposure in population studies on LAA.

In order to estimate the risk of LAA and identify its determinants, a follow-up study among approximately 600 laboratory animal workers is currently in progress in the Netherlands. In contrast to previous studies[11-14,17,18,22] on LAA, the analyses were based on sensitisation to rat allergens as well as on a combination of symptoms and sensitisation. Furthermore, allergen exposure is quantitatively assessed with a specifically developed immunoassay for RUA in personal airborne dust samples. This paper covers the base-line part of the study and describes the relationship between the prevalence rate of respiratory allergy to rats and exposure to rat urinary aeroallergens, which is controlled for host factors, like atopy, gender, and smoking.

MATERIALS AND METHODS

STUDY POPULATION

Employees from laboratory animal facilities of four universities (sites A, B, C and D),

two research institutes (sites E and F), one pharmaceutical company (site G) and students of a laboratory school (site H) participated in the study. A completed questionnaire as well as skin prick test (SPT) results were available for 540 participants[83]. Of these 540 participants, 458 (85%) worked with rats or had done so in the past. Eighty-two participants (15%), including 15 students from site H, had never worked with rats. These participants were used as internal reference group. For five of the 458 rat workers detailed information on occupational exposure to RUA was not available and these were left out of all analyses. Furthermore, four rat workers had only been working with blood or tissues derived from rats, 51 had worked with rats in the past and 398 had recently been working with living rats, i.e. during the preceding 12 months. All analyses were performed with recently exposed workers, because detailed information of exposure to rat allergens was only available for this period, and the internal reference group. The 4 workers who had been working with blood or tissues derived from rats were included in the internal reference group (n = 86).
Among laboratory animal workers five broad job titles could be distinguished: animal caretakers, animal technicians, scientists, scientific assistants, and supervisors. Over all sites a total of 20 different zones could be distinguished. A zone is an independent unit, which mostly consisted of several animal rooms and laboratories, and could be part of a building as well as a building itself[100].

EXPOSURE ASSESSMENT

Full-shift inhalable dust was sampled using IOM sampling heads (IOM, Edinburgh, Scotland). Each sampling head contained a polytetrafluoroethylene (Teflon) filter (Millipore; pore size 1.0 μm, ∅ 2.5 cm). RUA were eluted from the filters and assayed by sandwich enzyme immunoassay. Concentrations in test samples were expressed in nanograms of rat urinary protein equivalent (ng eq) per millilitre in which 1 ng eq was defined as the amount giving the same assay result as 1 ng protein of the standard. Two-thirds of the detection limit (0.15 ng eq/m^3) was assigned to samples in which RUA were not detectable. Details of air sampling and analysis of RUA are reported elsewhere (Chapter 2)[100].

QUESTIONNAIRE

A self-administered questionnaire was distributed before the medical survey (Chapter 5)[83]. Questions were asked about personal history of allergic symptoms to common allergens, history of allergic symptoms to laboratory animals, smoking history, working history, and intensity of contact with laboratory animals. Allergic symptoms due to working with rats were defined as the presence of self-reported allergic

symptoms (chest tightness (asthma), runny or sneezing nose, runny or itchy eyes, and itchy or red skin) during working hours, during or after contact with rats.

SKIN PRICK TESTING AND IGE ANTIBODIES

Five common allergens (house dust mites, grass pollen, tree pollen, cat fur and dog fur), two occupational allergens (rat urine and rat fur) and positive (histamine 10 mg/ml, in duplicate) and negative controls (PBS) were used for skin prick testing. All allergens were purchased from ALK Benelux (Houten, The Netherlands). SPTs were read after 15 minutes. A mean weal diameter of 3 mm or more for the common allergens and of 4 mm or more for the occupational allergens were regarded as positive, after subtraction of any response to the negative control (Chapter 5)[83].

Sera were stored at -20°C until IgE analysis. Specific IgE antibodies to RUA were measured by immunoassay (AlaSTAT; DPC, Apeldoorn, the Netherlands). Sera of class 2 or higher (≥ 0.7 Ku/l) were considered positive. Total IgE antibodies were measured with a sandwich enzyme immunoassay[113].

Sensitisation to rat allergens was defined as a positive SPT response to rat urinary or rat fur allergens, and/or the presence of specific serum IgE antibodies to rat urinary allergens. Workers were classified as allergic to rats if they reported allergic symptoms due to working with rats and if they were sensitised to rat allergens.

In a previous analysis of this population we identified elevated total serum IgE and type I sensitisation to cat or dog allergens as important risk factors of rat allergy (Chapter 5)[83]. These 'atopy associated risk factors' were defined as either self-reported cat or dog allergy, i.e. allergic symptoms (chest tightness (asthma), runny or sneezing nose, runny or itchy eyes, or itchy or red skin) during or after contact with cats or dogs; positive SPT response to cat or dog fur; and/or elevated total serum IgE (≥ 100 Ku/l).

STATISTICAL ANALYSIS

All statistical analyses were performed using SAS software (version 6.09). General linear regression models (SAS procedure GLM) were used in order to describe differences in log-transformed RUA concentrations by job title and zone. Crude prevalence rates were compared using the χ^2 (or Fisher's exact) test. The relationship between level of exposure and host factors, and the prevalence rate of sensitisation to rat allergens were analysed by multiple regression techniques. Prevalence rate ratios were calculated by means of a proportional hazards model (Cox's proportional hazard regression with SAS procedure PHREG)[114]. Statistical significance was reached at the $p < 0.05$ level (two-tailed).

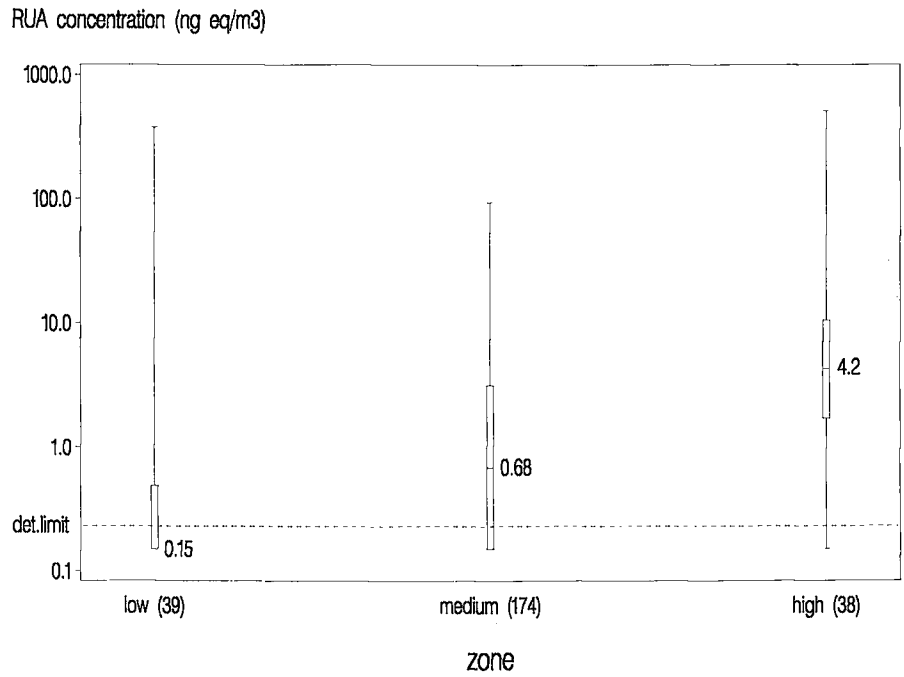


Figure 1. *Median (centre of box), 25th and 75th percentile (borders of box) and range (whiskers) of RUA concentrations (ng eq/m³), stratified by zone (low = site $C_{zone\ 1,2}$, $D_{zone\ 1}$ and $E_{zone\ 1}$; medium = site $A_{zone\ 1}$, $B_{all\ 7\ zones}$, $C_{zones\ 3,4}$, $E_{zone\ 2}$ and $G_{zone\ 1}$; high = site $D_{zone\ 2}$, $E_{zone\ 3}$ and $F_{zones\ 1,2}$) (n = 251).*

RESULTS

EXPOSURE

In order to estimate the exposure to RUA in the population of rat workers, full-shift inhalable airborne dust samples were taken in a representative sample of 87 workers from all job titles and zones. Each worker was sampled for one week on days when working with rats, resulting in a total of 251 personal full-shift airborne dust samples. The median RUA concentration of all samples was 0.76 ng eq/m^3. The exposure variability of the total samples set was large with a 175 fold difference between the 5th and 95th percentiles of the distribution. Of this variability 2%, 5% and 18% was explained by sampling day of the week, job title and zone, respectively. The analysis of variance revealed that the RUA concentrations were about twice as high on Mondays than on the other days of the week. Animal caretakers' exposure to RUA was about 2.5 times higher than that of the other rat workers. The most important determinant of RUA exposure was the zone in which the participant was working, with a 32-fold difference in median RUA level between the zone with the lowest and

highest exposure. The RUA levels of the workers in the 20 zones could be divided into three groups, low (workers of site $C_{zone\ 1,2}$, $D_{zone\ 1}$ and $E_{zone\ 1}$), medium (workers of site $A_{zone\ 1}$, $B_{all\ 7\ zones}$, $C_{zones\ 3,4}$, $E_{zone\ 2}$ and $G_{zone\ 1}$) and high (workers of site $D_{zone\ 2}$, $E_{zone\ 3}$ and $F_{zones\ 1,2}$). The median, 25th and 75th percentile and range of RUA levels of these exposure groups are presented in figure 1. The workers of most zones had comparable RUA exposures on days working with rats and were grouped as medium exposed workers (median RUA exposure level = 0.68 ng eq/m^3). The low exposed workers were working in zones in which the rats were housed in isolators or were only present in small numbers. In these zones RUA concentrations in 74% of the samples were below the detection limit, which resulted in a median RUA exposure level of 0.15 ng eq/m^3. The workers of the high exposure group had a more than average RUA exposure (median RUA exposure level = 4.2 ng eq/m^3). These workers worked in a special unit with high numbers of rats ($E_{zone\ 3}$) or in zones in which most of the work consisted of high exposed tasks, like handling contaminated bedding or handling rats.

Table 1. *Characteristics of rat workers and workers who had never worked with rats (internal reference group).*

	all rat workers	< 4 years of exposure	never worked with rats
n	398	118	86
female (%)	37.4¶	55.9	54.7
age (mean (SD))	35(10)	27(6)¶	33(11)
smoking* (%)	29.0§¶	33.9¶	11.6
atopy associated risk factors† (%)	39.6|	33.3§	33.7
allergic symptoms to rat allergens (%)	19.6¶	23.7¶	1.2
sensitised to rat allergens (%)	17.7¶	17.1§¶	3.5
rat allergy‡ (%)	13.4¶	15.4§¶	1.2

* when participants had quit smoking they were considered as non-smokers.

† allergic symptoms to cat or dog allergens, or a positive SPT response to cat or dog fur, or an elevated total serum IgE (≥ 100 Ku/l).

‡ allergic symptoms due to working with rats and sensitised to rat allergens.

§ 1 observation was missing

| 2 observations were missing

¶ $p < 0.01$ compared to the workers who never worked with rats

RAT ALLERGY

Among the 398 recently exposed rat workers, 78 (19.6%) reported at least one allergic symptom due to working with rats. A positive SPT response to rat allergens was

present in 17.3% of the rat workers and 11.1% had specific serum IgE antibodies to rat allergens. According to our definition, 70 (17.7%) workers were sensitised to rat allergens and 53 (13.4%) had rat allergy (table 1). In the internal reference group the prevalence rate of sensitisation to rat allergens was 3.5% and only one worker reported also allergic symptoms due to rat allergens (table 1). However, the allergic symptoms due to rat allergens could be under-reported in this group, because workers had no contact with living rats. Therefore, sensitisation to rat allergens was initially used as response variable in the exposure-response analyses.

Table 2. *Characteristics of participants recently exposed (preceding 12 months) to laboratory rats, grouped by zone.*

	zone working in		
	low	medium	high
n	63	313	22
years of exposure (mean (SD))	10(9.1)†	11(9.5)†	15(12)
median RUA level (ng eq/m^3)	0.15	0.68	4.2
allergic symptoms to rat allergens (%)	14.3	21.1	13.6
sensitised to rat allergens (%)	12.9*	18.9*	13.6
rat allergy (%)	8.1*	14.7*	9.1

* 1 observation was missing

† $p < 0.1$ compared to high exposure group

EXPOSURE-RESPONSE RELATIONSHIP

In a first approach to assess exposure-response relationships, the exposure to RUA was grouped according to zone in which the participant was working. The highest prevalence rate of sensitisation to rat allergens was found in medium exposed workers (18.9%) compared to low exposed workers (12.9%) and high exposed workers (13.6%; table 2). For rat allergy a similar pattern was found (table 2). Table 2 also shows that workers in the high exposure group had been working for a longer period in laboratory animal facilities and this could have biased exposure-response relationships. Similar relationships between exposure and prevalence rate of sensitisation to rat allergens were found when the workers were equally divided into three groups based on number of hours working with rats per week (low 16.1%; medium 19.8%; high 18.1%) or time-multiplied RUA exposure, i.e. number of hours working with rats per week multiplied by the median RUA level of the low, medium and high zone exposure groups as an estimate of weekly cumulative exposure (low 18.1%; medium 15.3%; high 19.8%). However, if prevalence rates were observed for

groups with limited years of exposure, i.e. less than 8, 6 or 4 years, prevalence rates increased with increasing exposure. Therefore, the exposure-response relations were studied in a group of rat workers with less than 4 years of exposure. Less than 4 years of working experience with laboratory animals was chosen as cut-off point, because most laboratory animal workers had a contract period of 4 years. Characteristics of this group are also presented in table 1.

Table 3. *Separate analysis of the association between various variables measuring the exposure to RUA and host factors, and sensitisation to rat allergens. The group of rat workers who had been working with rats for less than 4 years and the internal reference group were used in the analyses (n = 203).*

	n	prevalence rate (%)	prevalence rate ratio	95% CI
Exposure variable				
internal reference group (no exposure)	86	3.5	1	
zone working in				
low (0.15 ng eq/m^3)*	21	14.3	4.1	0.83 - 20
medium (0.68 ng eq/m^3)*	92	17.4	5.0	1.5 - 17
high (4.2 ng eq/m^3)*	4	25.0	7.2	0.75 - 69
hours working with rats				
0 - 2.5 hours per week	49‡	8.2	2.3	0.52 - 10
2.5 - 9 hours per week	30‡	13.3	3.8	0.86 - 17
9 or more hours per week	38‡	31.6	9.1	2.6 - 32
time-multiplied RUA exposure†				
> 0 till 1 hours weekly ng eq/m^3 (low)	40‡	7.5	2.2	0.43 - 11
≥ 1 till 6 hours weekly ng eq/m^3 (medium)	43‡	11.6	3.3	0.80 - 14
≥ 6 hours weekly ng eq/m^3 (high)	34‡	35.3	10.1	2.9 - 36
Host factors				
atopy associated risk factors				
none	135	2.2	1	
at least one	68	29.4	13.2	3.9 - 45
gender				
male	91	16.5	1	
female	112	7.1	0.43	0.18 - 1.0
smoking				
non-smoker	154	8.4	1	
smoker	49	20.4	2.4	1.1 - 5.5

* median RUA levels for each group

† the median RUA levels for the zone groups (low, medium and high) were multiplied with the number of hours per week spent working with rats.

‡ the exposed workers were equally divided into three groups. The number of workers in each group could differ slightly, because of workers with similar values.

In the restricted population an exposure-response relationship was visible. The prevalence rates of sensitisation to rat allergens in the low, medium and high exposure zone groups were about 4.1, 5.0 and 7.2 times higher, respectively, compared to the prevalence rate found in the internal reference group (table 3). A steeper exposure-response relationship was found when grouping was based on number of hours working with rats per week (table 3). The strongest and statistically most significant exposure-response relationship was found for the time-multiplied RUA exposure (table 3). The prevalence rate ratio increased with the increasing time-multiplied RUA exposure and was 2.2 for low exposure (> 0 till 1 hours weekly ng eq/m^3), 3.3 for medium (≥ 1 till 6 hours weekly ng eq/m^3), and 10 for high exposure (≥ 6 hours weekly ng eq/m^3). The difference in prevalence rate between high and low exposed workers in an internal comparison was also statistically significant.

Relations between possible confounders or modifying factors and sensitisation to rat allergens are also presented in table 3. The atopy associated risk factors were strongly associated with sensitisation to rat allergens, prevalence rate ratio of 13 (95% confidence interval (CI) 3.9 - 45). Furthermore, sensitisation was about 2.3 times more prevalent among men than among women and about 2.4 times more prevalent among smokers (ex-smokers were considered as non-smokers). The associations between sensitisation to rat allergens and smoking were statistically significant. There was no association between age and prevalence rate of sensitisation to rat allergens.

The time-multiplied RUA exposure and the modifying factors were studied in a multiple regression analysis (table 4). Atopy associated risk factors appeared to be a strong effect modifier: the effect of exposure to RUA on the prevalence rate of sensitisation to rat allergens differed clearly between the workers with and without the atopy associated risk factor. After stratification, prevalence rate ratios for the workers with an atopy associated risk factor were 7.3, 9.5 and 15 for the low, medium and high time-multiplied RUA exposure groups, respectively. Among workers with no atopy associated risk factors the exposure-response relationship was much less pronounced, showing only a slight increase of the prevalence rate ratios of sensitisation to rat allergens with increasing time-multiplied RUA exposure level. In the group of sensitised rat workers, 90% reported allergic symptoms due to rat allergens. When a more strict definition of allergy to rats was used, i.e. the presence of reported symptoms and sensitisation to rat allergens as also used elsewhere[83], the exposure-response relationship within the group of rat workers became even steeper for those with atopy associated risk factors (table 4). The effect of gender and smoking on the prevalence rate of sensitisation to rat allergens or rat allergy was still visible in the multiple regression analysis. However, both associations were not statistically significant.

Table 4. *Multiple regression analyses of relationship between prevalence rate ratios of sensitisation to rat allergens or rat allergy, and time-multiplied RUA exposure. The analyses were controlled for atopy associated risk factors, smoking and gender (n = 203).*

			Sensitisation to rat allergens		rat allergy	
		n	prevalence rate ratio	95% CI	prevalence rate ratio	95% CI
time-multiplied exposure	**atopy associated risk factors**					
never worked with rats	no distinction	86	1.0		1.0	
low	none	29	-*	---	-*	---
medium	none	31	0.84	0.086 - 8.2	2.5	0.16 - 41
high	none	18	1.3	0.14 - 13	3.9	0.24 - 64
low	at least one	11	7.3	1.5 - 37	7.5	0.46 - 120
medium	at least one	12	9.5	2.1 - 43	29	3.2 - 260
high	at least one	16	15	4.0 - 56	42	5.2 - 330
gender, female versus male			0.58	0.24 - 1.4	0.42	0.15 - 1.2
smoking			1.6	0.69 - 3.9	1.8	0.71 - 4.6

* no prevalence rate ratio could be calculated, since there were no sensitised workers in this group

DISCUSSION

Our study clearly shows that a relationship between exposure to rat urinary aeroallergens and prevalence rate of allergy to rats exists. The exposure-response relationship became visible after restriction of the analyses to the group of workers who have been working with laboratory animals for a limited period of years. The exposure-response relationship is most pronounced in workers with allergic symptoms to cat or dog allergens, or positive SPT response to cat or dog fur, or elevated total serum IgE.

In laboratory animal facilities the variability of exposure is considerable[100]. In contrast to the study of Nieuwenhuijsen and co-workers[101], the zone in which the participant was working explained a larger percentage of the variability in RUA levels than job title. However, in the study of Nieuwenhuijsen and co-workers[101] exposure measurements were performed in two sites, whereas we performed our measurements in seven sites. The large variability in exposure between zones or sites may be due to differences in level of contamination of bedding in cages, varying numbers of animals of different sex and age used, way in which tasks are performed, and type and varying

levels of ventilation at the sites[100]. An exposure-response relationship could be demonstrated when the workers were grouped on the basis of RUA measurements, i.e. zone working in. Besides grouping based on RUA measurements, hours working with rats per week, which has been determined by questionnaire, was also associated with sensitisation to rat allergens. However, this relatively crude estimate of exposure did not differentiate completely between high and low exposed workers. The combination of the actual RUA levels grouped by zone and hours working with rats per week, i.e. time-multiplied RUA exposure, resulted in the most pronounced exposure-response relationship compared to the relationships found by the other grouping schemes.

A relationship between the prevalence rate of respiratory allergic symptoms to rats and exposure to rat urinary aeroallergens has also been found in two other studies[18,22]. In both studies the exposure assessment was also performed by measuring rat allergen concentrations. Kibby and colleagues[18] also found that time-multiplied exposure gave the best relationship with LAA symptoms. In contrast to our study, the number of air dust samples was limited. In the study of Cullinan and colleagues[22] a large number of full-shift personal air measurements were taken. However, they found an exposure-response relationship for allergic skin symptoms, but not for chest, eye, nasal symptoms, and sensitisation.

The existence of exposure-response relationships between level of exposure and sensitisation to aeroallergens has also been suggested in a limited number of studies on other aeroallergens. In occupational studies more workers were sensitised to α-amylase[29] or *Bacillus subtilis* enzymes[121] in groups with high exposure. In these populations the exposure-response relationships were also more pronounced in atopic workers.

A relationship between the prevalence rate of sensitisation to rat allergens and exposure to rat urinary aeroallergens can be biased by selection, inaccurate information about the presence of an allergy to rats and confounding. In our population strong indicators were found that duration of exposure had an effect on the prevalence rate of sensitisation and symptoms to rat allergens. Sensitisation and symptoms to rat allergens were less prevalent among workers with the highest exposure. In addition, these workers were exposed to rat allergens during a longer period (table 2). Two possible explanations for this observation are a healthy worker effect or so-called tolerance induction. Workers with LAA can be selected out of the workforce or be transferred to jobs with less exposure, i.e. healthy worker effect. High exposed workers can probably less easily modify their work to avoid animal contact if they develop symptoms, and might leave the site at a different rate than the lower exposed workers. The presence of a healthy worker effect was also assumed to occur

in one other cross-sectional study on LAA[17]. At the same time, longer duration and increased levels of exposure to an allergen by inhalation may induce tolerance to the allergen. However, to our knowledge there is no evidence in the literature of tolerance induction by inhalation. Conclusive answers about the presence of a healthy worker effect or tolerance induction can only be given after analysis of follow-up data.

In our study we accounted for the effect of duration of exposure by restricting the population to workers with less than 4 years of working experience with laboratory animals. Four years was chosen because most laboratory animal workers were PhD-students with a contract period of 4 years. However, this cut-off point is arbitrary, but analyses with a restricted population using 3 or 5 years as cut-off point gave similar results as shown in table 3 and 4. One other study on LAA[22] also restricted the population to newly exposed workers, and also used 4 years of exposure as cut-off point.

Inaccurate information about the presence of allergy to rats could also bias the exposure-response relationship. The studies of Kibby and colleagues[18] and Cullinan and colleagues[22] as well as all other cross-sectional studies on LAA used the presence of allergic symptoms as criterion for LAA. As published elsewhere[83], allergic symptoms after contact of non-sensitised workers with rats may be the result of a non-specific hyperresponsiveness to animal-derived or other agents, such as dust, disinfectants or ammonia, which are present simultaneously. We therefore decided to use sensitisation to rat allergens as endpoint. Of the sensitised rat workers only 2 workers were without symptoms and therefore a similar exposure-response relationship was found when rat allergy, i.e. the presence of reported symptoms and sensitisation to rat allergens, was used as measure of response. In fact, using rat allergy as measure of response resulted in a steeper exposure-response relationship.

The relationship between the prevalence rate of sensitisation to rat allergens and exposure to rat urinary aeroallergens can be modified by host factors. Host factors related to atopy are associated with LAA[11,12,14,17,19,21,22,35]. In a previous analysis of this population we identified that of these factors self-reported allergy or sensitisation to cats or dogs, and elevated total serum IgE were important risk factors of rat allergy[83]. In the present study we noticed that the effect of exposure on the prevalence rate of sensitisation to rat allergens clearly varied between workers with and without an atopy associated risk factor. The exposure-response relationship was much steeper among workers with an atopy associated risk factor. The atopy associated risk factor should therefore be considered as effect modifier of exposure.

In previous studies on occupational allergy, the effect of gender received relatively little attention. Only two studies have previously studied the effect of gender on the

prevalence rate of LAA[14,72]. In contrast to our study, in which sensitisation to rat allergens was more prevalent in men, no differences in prevalence rates had been found between men and women. However, general population studies have found sensitisation to common allergens to be more prevalent in men[73-77]. In our study the atopy associated risk factors were also more prevalent in male than female rat workers, 38.5% to 30.8%, respectively. This higher prevalence rate of atopy associated risk factors as well as the higher 'time-multiplied RUA exposure' levels for men, 9.0 hours weekly ng eq/m^3 versus 4.6 hours weekly ng eq/m^3 for women, could account for the higher prevalence rate of sensitisation to rat allergens in men. However, after correcting for level of exposure, the atopy associated risk factors and smoking, sensitisation to rat allergens was still about twice as prevalent in men (table 4). This difference was not statistically significant because of the small sample size, but it suggests that gender is an independent risk factor of LAA.

In our study sensitisation to rat allergens was about 1.6 times more prevalent among smokers. However, this finding was not statistically significant. There is conflicting evidence regarding the role of smoking in sensitisation to occupational allergens. Some studies[22,71,72,117-119] have found sensitisation to occupational allergens to be more common in smokers, while several other studies[10,12,14,15] failed to demonstrate this relationship or even found a negative association[122]. An explanatory factor might be the cross-sectional design of most studies. It is possible that smoking habits were influenced by the development of symptoms or that subjects allergic to common allergens would less easily pick up the habit of smoking. Whatever the role of gender and smoking is in the relation between rat allergen exposure and subsequent sensitisation, gender and smoking remain relatively weak effect modifiers compared to exposure to rat urinary aeroallergens and atopy related host factors.

CHAPTER 7

WORK RELATED CHANGES IN PEAK EXPIRATORY FLOW AMONG LABORATORY ANIMAL WORKERS

ABSTRACT

Background: Laboratory animal workers are at risk of developing allergic symptoms, of which asthmatic symptoms are the most severe.

Objective: To study the relationship between allergic symptoms, and PEF variability and changes in PEF due to working with rats.

Methods: Several indices were used on the basis of amplitude or differences in PEF between days with and without rat allergen exposure. Of the 398 rat workers, 73% completed PEF readings on at least 9 days, of which 208 workers had PEF readings on working days with and without contact with animals.

Results: The prevalence rate of allergic symptoms caused by rats was 17.3%. Chest tightness was reported by 6.7% of the workers. The lowest PEF of workers who reported chest tightness caused by rats, decreased significantly on days working with laboratory animals (Δ $PEF_{min\text{-}min}$ = -7.3 l/min), compared to the workers without symptoms (2.2 l/min). This effect was more pronounced among workers with chest tightness several hours after working with rats (Δ $PEF_{min\text{-}min}$ = -11.6 l/min). Multiple regression analyses showed that beside chest tightness several hours after working with rats, only the presence of allergic symptoms to pets was associated with a Δ $PEF_{min\text{-}min}$. In addition, workers with chest tightness were also more likely to have a higher PEF variability than workers without chest tightness. However, no difference in PEF variability between days with and without animal contact was observed.

Conclusions: This study shows that the PEF of workers who reported chest tightness due to working with rats, decreased significantly on days working with laboratory animals.

---

by Albert Hollander, Dick Heederik and Bert Brunekreef, submitted

INTRODUCTION

Laboratory animal workers are at risk of developing work related allergic asthma. Cross-sectional epidemiological studies among laboratory animal workers have reported prevalence rates of asthmatic symptoms ranging from 4-12%[11-13,17,22,83]. Asthmatic symptoms are mostly accompanied by other respiratory symptoms and are considered as end-stage of laboratory animal allergy (LAA)[12,17,20,22].

Occupational asthma can be demonstrated by recording the peak expiratory flow (PEF) several times a day on days away from and at work[123-126]. In clinical practice, several investigators have shown how visual inspection of PEF records can prove useful in identifying individuals with occupational asthma[124,127]. However, workers who are being referred to clinical practices, have mostly severe asthma and their PEF records usually show clear differences between working and non-working days. It is not clear to what extent monitoring of PEF is a useful tool to detect the presence of LAA in epidemiological surveys in workers who have not (yet) been referred to a clinical practice.

In large scale epidemiological studies the visual inspection of PEF records is not practical and a numerical expression of PEF variability or changes in PEF is to be preferred. The amplitude percentage mean, defined as the daily maximal PEF minus the minimal PEF expressed as a percentage of the daily average, has been suggested as index of PEF variability[128]. This amplitude percentage mean has been applied in a limited number of occupational studies[129-131], in which it was shown to be associated with exposure to grain dust, polyvinylchloride and toluene diisocyanate. Serial PEF recording has also been used to study across work shift decline in PEF in a population exposed to endotoxin[132]. However, none of these studies have used the differences in PEF variability or decline in PEF between days away from and at work as tool to study occupational asthma.

In a cross-sectional study among 540 laboratory animal workers, relationships between exposure to rat urinary aeroallergens (RUA) and prevalence rate of sensitisation to rat allergens and allergic symptoms due to working with rats were found[86]. Furthermore, cat and dog allergy, and an elevated total IgE level were found to be strong risk factors of LAA[83,86]. As part of this study, the workers recorded their PEF over a period of about two weeks. This paper describes the PEF variability and changes in PEF and their relationships with allergic symptoms due to working with rats and sensitisation to rat allergens. Several indices of PEF variability and changes in PEF were used, all focusing on the differences between days with and without exposure to RUA. In addition, we studied the associations between these indices and average level of exposure to RUA, smoking, gender and atopy.

MATERIALS AND METHODS

STUDY POPULATION

Employees from laboratory animal facilities of four universities, two research institutes, one pharmaceutical company and students of a laboratory school participated in the study. A completed questionnaire as well as skin prick test (SPT) results were available for 540 participants (Chapter 5)[83]. Of these 540 participants, 458 (85%) worked with rats or had done so in the past. For 5 of the 458 rat workers no detailed information on occupational exposure to RUA was available and these were excluded from the analyses. Furthermore, 4 rat workers had only been working with blood or tissues derived from rats, 51 had worked with rats in the past and 398 had recently been working with living rats, i.e. during the preceding twelve months. Thus, analyses were performed with 398 recently exposed workers, because detailed information of exposure to RUA was only available for this period.

QUESTIONNAIRE

The self-administered questionnaire contained questions about personal history of allergic symptoms due to common allergens, smoking history, occupational history and intensity of contact with laboratory animals (Chapter 5)[83]. In addition, questions were asked about the history of allergic symptoms due to working with rats during working hours as indicator of an immediate response: 'Do you have allergic symptoms during working hours, after contact with rats?'. If positive, questions were asked on type of symptoms (chest tightness (asthma), runny or sneezing nose, runny or itchy eyes, and itchy or red skin). A similar question was asked with respect to symptoms occurring several hours after work as indicator of a late response: 'Do you have allergic symptoms several hours after finishing work, due to contact with rats?'. In this paper having symptoms due to working with rats is defined as an immediate and/or late response, unless otherwise stated.

SPIROMETRY

The FVC, FEV_1 and also the PEF were measured with a dry rolling seal Vicatest V (Jeager, Breda, The Netherlands). Measurements were performed according to the lung function protocol of the European Community for Steel and Coal[133].

PEF RECORDING

Each participant was given a mini-Wright peak flow meter and proper use of the meter was demonstrated immediately following administration of the questionnaire and performing the spirometry. Participants were asked to record PEF during the two

weeks following the medical survey. However, the diary could contain up to 20 days. Participants were asked to record PEF on four occasions during working days: on waking, at lunch time, just after the working day and at bedtime[134]. During weekend/holidays the participants had to record PEF on three occasions: on waking, at lunch time and at bedtime. On each occasion they were asked to blow after maximal inspiration three times into the peak flow meter and record all readings. Measurements were made in the standing position and the scale was read to the nearest scale mark (5 l/min). On each working day contact with laboratory animals (yes/no) was recorded.
A day started with the first reading at work (after 9.30 am), continuing for the next 24 hours, so that the first reading after waking is included in the exposure period of the previous day[123]. The highest of the three attempts was used for analysis. All PEF records were plotted in two graphs to detect obvious data errors. The first graph showed the maximum, minimum and mean of each day's PEF and the second graph showed the maximal PEF of each measurement. Of each individual series the first day was left out, because of the possibility of a learning effect. Only participants who had at least three readings per day for at least nine days were used for the analyses presented in this paper.
Several indices were used on the basis of the amplitude (AMP) of the PEF or the differences in PEF (Δ PEF) between working days with and without laboratory animal contact (figure 1). The AMP was calculated for each day and averaged for weekend days ($AMP_{weekend}$), working days without laboratory animal contact ($AMP_{no\ animal\ contact}$) and working days with laboratory animal contact ($AMP_{animal\ contact}$). The Δ AMP represents the difference between the $AMP_{animal\ contact}$ and the $AMP_{no\ animal\ contact}$. Furthermore, two different Δ PEF indices were used, Δ $PEF_{max\text{-}max}$ and Δ $PEF_{min\text{-}min}$ (figure 1). All indices were expressed in l/min.

EXPOSURE ASSESSMENT

In order to estimate the exposure to RUA in the population of rat workers, personal full-shift inhalable dust was sampled and assayed on rat urinary allergen content by a sandwich enzyme immunoassay (Chapter 2)[100]. The dust samples were taken in a representative sample of 87 workers from all job titles and working zones. Each worker was sampled for one week on days when working with rats, resulting in a total of 251 personal full-shift airborne dust samples. On the basis of these measurements the zones were divided into three groups. The average RUA levels of these groups were combined with the hours working with rats per week as reported in the questionnaire, which resulted in an average 'time-multiplied RUA exposure' for each worker (Chapter 6)[86]. Finally, the workers were grouped on the basis of their 'time-

multiplied RUA exposure'.

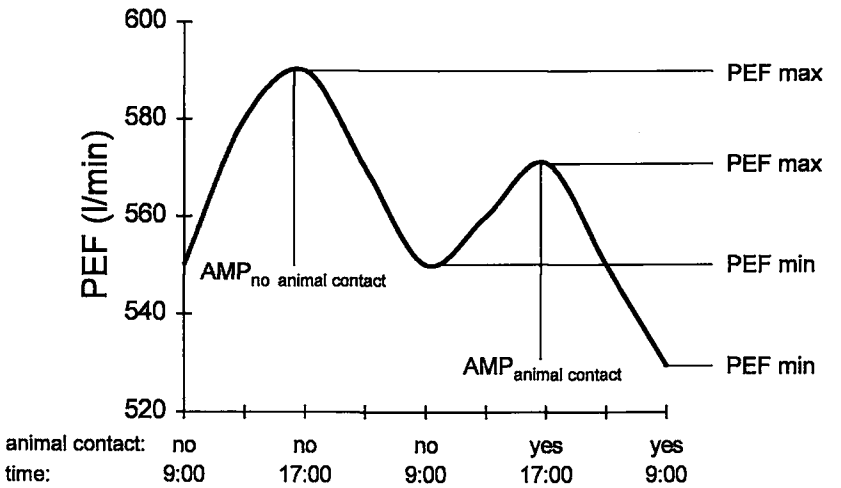


Figure 1. *The various PEF indices used:*

$AMP_{no\ animal\ contact}$	*amplitude of PEF (PEF_{max} - PEF_{min}) on a working day without laboratory animal contact, averaged over all days*
$AMP_{animal\ contact}$	*amplitude of PEF (PEF_{max} - PEF_{min}) on a working day with laboratory animal contact, averaged over all days*
$AMP_{weekend}$	*amplitude of PEF (PEF_{max} - PEF_{min}) on a weekend or holiday, averaged over all days*
ΔAMP	$AMP_{animal\ contact}$ *minus* $AMP_{no\ animal\ contact}$
$\Delta PEF_{max\text{-}max}$	PEF_{max} *averaged over all working days with laboratory animal contact minus* PEF_{max} *averaged over all working days without laboratory animal contact*
$\Delta PEF_{min\text{-}min}$	PEF_{min} *averaged over all working days with laboratory animal contact minus* PEF_{min} *averaged over all working days without laboratory animal contact*

SKIN PRICK TESTING AND IGE ANTIBODIES

Five common aeroallergens (house dust mites, grass pollen, tree pollen, cat fur and dog fur), two occupational allergens (rat urine and rat fur) and positive (histamine 10 mg/ml, in duplicate) and negative controls (phosphate-buffered saline, PBS) were used for skin prick testing. In the sera specific IgE antibodies to RUA were measured by immunoassay (AlaSTAT; DPC, Apeldoorn, the Netherlands, Chapter 5)[83].

Sensitisation to rat allergens was defined as a positive SPT response to rat urinary or rat fur allergens, and/or the presence of specific serum IgE antibodies to rat urinary allergens.

STATISTICAL ANALYSIS

All statistical analyses were performed using SAS software (version 6.09). Prevalence rates were compared using either the χ^2 or the Fisher's exact test. For testing group mean differences Student's two-sample t-test was used. The distribution of the AMP and Δ PEF indices were not clearly normally or log-normally distributed. Therefore, the median levels of these distributions were presented and group median differences were tested by using the nonparametric Wilcoxon rank-sum test for two levels.
Relationships between allergic symptoms due to working with rats, sensitisation to rat allergens, average level of exposure to RUA and host factors, and the PEF indices were studied by linear multiple regression analyses (PROC REG).

RESULTS

POPULATION CHARACTERISTICS

Of the 398 workers who had recently been working with rats, i.e. during the preceding twelve months, 289 (73%) completed PEF readings on at least nine days. In this group of workers the average number of days was 13, range 9 to 19 days. Seventy one workers (18%) returned an incomplete diary and 38 workers (10%) did not return their diary at all. The workers of whom no properly completed PEF record was available were significantly younger (table 1). These workers also smoked more often and reported more allergic symptoms due to working with rats. However, these differences were not statistically significant.
Since we observed a difference between $AMP_{weekend}$ and $AMP_{no\ animal\ contact}$ (table 1), which might be attributed to differences in time of the measurements, we only used the PEF readings on working days with and without animal contact in all further analyses. Two hundred and eight rat workers had PEF readings on both working days with and without animal contact (table 1). Among these workers, the prevalence rate of sensitisation to rat allergen and the prevalence rate of allergic symptoms (chest, eye, nose and/or skin) during or after working with rats was 18.4% and 17.3%, respectively. Thirteen percent of the rat workers was both sensitised and symptomatic. Chest tightness during or after handling rats was reported by 6.7% of the workers. Of these workers with chest tightness 86% were sensitised to rat allergens.

Table 1. Characteristics of rat workers, stratified by PEF readings

	PEF readings		PEF readings on working days with and without animal contact
	no	yes	
n	109	289	208
female, %	34.9	38.4	42.8
age, mean ± SD	32.5(9.7)	35.3(9.6)‡	34.6 ± 9.3
current smoker, %	35.8	26.4†	24.6†
allergic symptoms due to rats, %	25.7	17.3	17.3
sensitised to rat allergens, %	17.6†	17.7†	18.4†
rat allergy*, %	15.7†	12.5†	13.0†
PEF variability, median in l/min			
$AMP_{weekend}$		25.0(n=282)	25.0(n=204)
$AMP_{no\ animal\ contact}$		30.0(n=256)	30.0(n=208)
$AMP_{animal\ contact}$		30.0(n=241)	29.6(n=208)
spirometry, percentage predicted[133]			
FVC	102(n=108)	104(n=285)	103(n=205)
FEV_1	101(n=108)	101(n=285)	100(n=205)
PEF	113(n=108)	112(n=285)	111(n=205)

* allergic symptoms due to working with rats and sensitised to rat allergens

† 1 observation was missing

‡ $p < 0.05$ compared to the workers with no PEF-readings

PEF INDICES

The PEF indices, stratified by sensitisation to rat allergens or allergic symptoms due to working with rats, are presented in table 2. Both $AMP_{no\ animal\ contact}$ and $AMP_{animal\ contact}$ were significantly higher for sensitised rat workers compared to non-sensitised workers. However, the amplitudes did not differ between working days with and without animal contact (Δ AMP = 0). More contrast in the indices of PEF variability was found after stratifying for allergic symptoms due to rat allergens (table 2). The $AMP_{no\ animal\ contact}$ and $AMP_{animal\ contact}$ were both significantly higher for rat workers with chest tightness due to working with rats, compared to workers without symptoms. The amplitudes of workers with other allergic symptoms, i.e. nose, eye or skin symptoms, were only slightly higher compared to the amplitudes of the workers without symptoms. The Δ AMP was higher for workers with chest tightness compared to the other workers. However, these differences were not statistically significant.

The Δ $PEF_{min\text{-}min}$ and Δ $PEF_{max\text{-}max}$ were both significantly decreased among the workers with chest tightness compared to the other workers (table 2). Interestingly, the Δ $PEF_{min\text{-}min}$ and Δ $PEF_{max\text{-}max}$ of the workers with chest tightness were decreased by a similar magnitude, -7.3 and -6.7 l/min, respectively. The indices presented in table 2

suggest that among the workers with chest tightness the mean PEF was lower but the mean amplitude was similar on working days with laboratory animal contact compared with working days without animal contact. Despite the significant differences of the median values of the various indices between groups, the variation of the indices is large within each of these groups (figure 2).

Table 2. *The PEF indices* stratified by sensitisation to rat allergens and allergic symptoms (chest, eye, nose and/or skin) due to working with rats.*

	sensitisation to rat allergens†		symptoms due to working with rats		
	no	yes	no	chest tightness	other
n	169	38	171	14	23
PEF, l/min ± SD	550 ± 96	587 ± 89	549 ± 97	544 ± 97	613 ± 77|
no. of working days without animal contact ± SD	4.4 ± 2.6	4.3 ± 3.2	4.5 ± 2.7	3.5 ± 2.3	4.1 ± 3.2
no. of working days with animal contact ± SD	5.6 ± 3.2	5.7 ± 3.3	5.5 ± 3.3	5.8 ± 2.4	6.3 ± 3.4
indices of PEF variability					
$AMP_{no\ animal\ contact}$, l/min	28.0	33.2§	29.0	35.3‡	30.0
$AMP_{animal\ contact}$, l/min	29.0	35.2‡	28.8	35.2‡	33.3
Δ AMP, l/min	0.00	0.00	-0.24	3.5	0.00
indices of changes in PEF					
$\Delta\ PEF_{max-max}$, l/min	2.5	-2.4‡	2.5	-6.7§	1.7
$\Delta\ PEF_{min-min}$, l/min	1.9	-0.71	2.2	-7.3§	4.0

* the median is presented

† 1 observation missing

‡ $p < 0.05$ (one-tailed) compared to the non-sensitised group or group with no symptoms

§ $p < 0.01$ (one-tailed) compared to the non-sensitised group or group with no symptoms

| $p < 0.05$ compared to group with no symptoms and the group with chest tightness

Of the 14 workers with chest tightness, 10 workers reported chest tightness several hours after working with rats, which was used as an indicator of a late asthmatic response. The indices of PEF variability ($AMP_{no\ animal\ contact}$ = 39.2 l/min, $AMP_{animal\ contact}$ = 37.0 l/min) and indices of changes in PEF ($\Delta\ PEF_{min-min}$ = -11.6 l/min, $\Delta\ PEF_{max-max}$ = -10.9 l/min) were all higher respectively lower compared to the values presented in table 2, suggesting more pronounced effects on PEF in workers with a late asthmatic response.

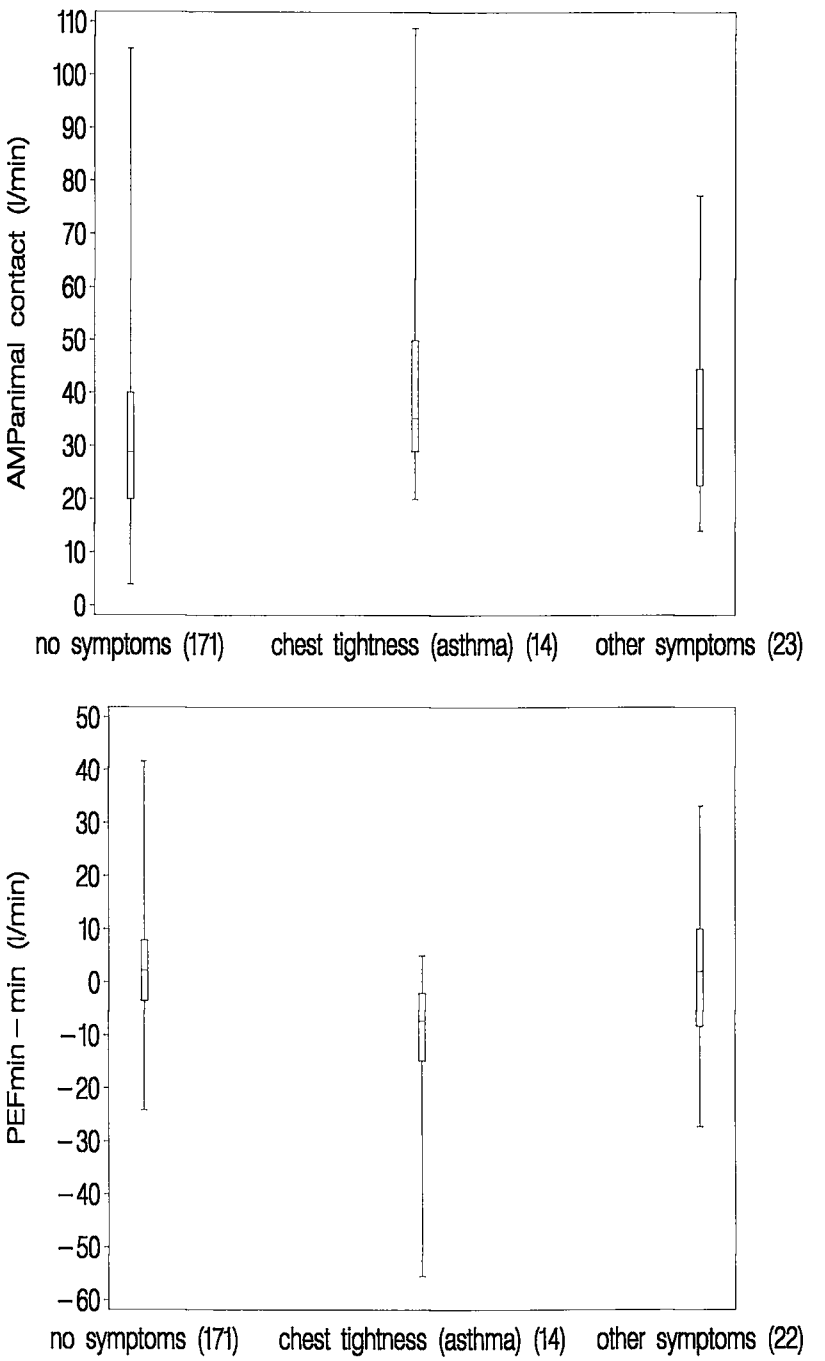


Figure 2. *Median (centre of box), 25th and 75th percentile (borders of box) and range (whiskers) of $AMP_{animal\ contact}$ (l/min) (top, n = 208) and $\Delta\ PEF_{min\text{-}min}$ (l/min) (bottom, n = 207, one was not plotted because of a very high level), both stratified by allergic symptoms due to working with rats (N = 208).*

Table 3 shows the associations between the PEF indices and average exposure level to RUA, and several host factors. The $AMP_{no\ animal\ contact}$ and $AMP_{animal\ contact}$ were significantly elevated among workers in the group with high average RUA exposure compared to these indices among the workers in the low exposure group. No effect of the average level of exposure was found on the Δ AMP and Δ PEF indices. The $AMP_{no\ animal\ contact}$ and $AMP_{animal\ contact}$ were both elevated for smokers, 34.0 and 33.6 l/min for smokers and 27.4 and 28.8 l/min for non-smokers, respectively. However, this difference was statistically significant only for the $AMP_{no\ animal\ contact}$. Again, no effect was found on the Δ AMP and Δ PEF indices. Of the host factors related to atopy, a positive SPT reaction to at least one of the five common allergens was significantly associated with the $AMP_{no\ animal\ contact}$ and $AMP_{animal\ contact}$. These differences were larger when only the SPT responses to cat and dog fur were taken into account, 35.0 and 34.4 l/min (table 3). Among workers with positive SPT responses to grass pollen, tree pollen or house dust mite allergens but negative SPT responses to cat and dog fur, these indices were similar (both 27.5) as among workers with no response to any common allergen (27.3 and 27.9 l/min, respectively). Similar but less pronounced results were found for personal history of allergic symptoms to common allergens as reported in the questionnaire. On the other hand workers with allergic symptoms to pets had a significantly decreased $\Delta\ PEF_{min\text{-}min}$ when compared to all other workers (table 3). Of the workers with allergic symptoms to pets, the $\Delta\ PEF_{min\text{-}min}$ was more decreased among workers who were also sensitised to rat allergens, -8.8 l/min, compared to -2.8 l/min for non-sensitised workers. None of the PEF indices were associated with gender, age, height or mean PEF.

The variables presented in table 2 and 3 were used in multiple regression analyses with the PEF indices as dependent variables (table 4). Sensitisation to rat allergens, symptoms due to working with rats, average level of exposure to RUA and several host factors explained less than 12% of the variation of the indices. Similar to the results presented in table 2 and 3, the variables tested resulted in an increase in $AMP_{no\ animal\ contact}$ as well as in $AMP_{animal\ contact}$. However, of all variables tested only a SPT positive response to cat and/or dog fur was statistically significant associated with the $AMP_{no\ animal\ contact}$. In contrast, chest tightness due to rat allergens several hours after working with rats was strongly associated with the Δ PEF indices. All other variables showed no relation with the Δ PEF indices, except for the presence of allergic symptoms to pets, which was associated with a decrease in $\Delta\ PEF_{min\text{-}min}$.

Table 3. *Separate analyses of the associations between the PEF indices (l/min)* and average level of exposure to RUA, and various host factors (n = 208).*

Fout! Bladwijzer niet gedefinieerd.		n	$AMP_{no\ animal\ contact}$	$AMP_{animal\ contact}$	Δ AMP	$\Delta PEF_{max\text{-}max}$	$\Delta PEF_{min\text{-}min}$
average RUA exposure	low	70	28.6	28.5	-0.83	1.7	2.0
	medium	75	27.5	27.5	0.83	2.0	0.75
	high	63	30.0‡|	34.2‡	0.00	1.7	2.7
smoking†	no	156	27.4	28.8	0.83	2.0	1.9
	yes	51	34.0§	33.6	-1.3	0.00	0.17
SPT^- to all five common allergen		126	27.3	27.9	-0.23	2.4	1.1
SPT^+ to cat and/or dog fur		51	35.0§	34.4‡	0.00	0.00	1.9
SPT^+, but SPT^- to both cat and dog fur		31	27.5|	27.5	1.5	3.3	2.7
no history of allergic symptoms to common allergens		149	28.0	28.9	0.00	2.5	2.3
allergic symptoms to pets		23	30.0	31.1	2.9	-0.83	-5.0‡
symptoms to common allergens, but no symptoms to pets		36	31.7	34.4	0.42	0.00	1.6|

* the median is presented

† 1 observation missing

‡ $p < 0.05$ (one-tailed) compared to 'no' or first group

§ $p < 0.01$ (one-tailed) compared to 'no' or first group

\| $p < 0.05$ (one-tailed) compared to medium exposure, SPT^+ to cat and/or dog fur, or group with allergic symptoms to pets

Table 4. *Regression coefficients and standard errors of multiple regression analyses with the PEF indices (l/min) as dependent variable and sensitisation, allergic symptoms, exposure level and various host factors as independent variables (n = 205*).*

Fout! Bladwijzer niet gedefinieerd.	ß (SE) of various indices of PEF variability			
	$AMP_{no\ animal\ contact}$	$AMP_{animal\ contact}$	$\Delta\ PEF_{max\text{-}max}$	$\Delta\ PEF_{min\text{-}min}$
R^2, %	9.9	7.5	8.7	11.1
constant	27.0(1.5)†	29.0(1.6)†	4.7(1.3)†	2.6(1.1)†
chest tightness several hours after working with rats	6.8(5.4)	6.8(5.9)	-16.0(4.6)†	-16.0(4.1)†
sensitisation to rat allergens	1.6(3.3)	4.3(3.6)	1.9(2.8)	-0.87(2.5)
exposure to RUA, high versus medium/low	3.4(2.4)	2.1(2.6)	-0.63(2.0)	0.63(1.8)
smoking	3.6(2.5)	1.9(2.8)	-2.3(2.1)	-0.60(1.9)
SPT‡ to cat or dog fur	6.9(2.8)†	4.7(3.1)	-1.0(2.4)	1.2(2.2)
allergic symptoms to pets	2.2(3.5)	3.3(3.8)	-3.9(2.9)	-5.0(2.6)‡

* 3 workers deleted because blood sample was missing, information on smoking habit was missing or of outlying variables

† $p < 0.01$ (one-tailed)

‡ $p < 0.05$ (one-tailed)

DISCUSSION

This study shows that the PEF of workers who reported chest tightness due to working with rats decreased significantly on days working with laboratory animals. This effect was more pronounced among workers with a late asthmatic response, i.e. the presence of chest tightness several hours after working with rats. In addition, workers with chest tightness were also more likely to have a higher PEF variability than workers without chest tightness, even if these workers without chest tightness experienced skin, eye or nose symptoms due to working with rats. No difference in PEF variability between days with and without contact with laboratory animals was observed.

Variation of PEF in time has shown to be a useful marker of reversible airway obstruction due to exposure to environmental hazards. In clinical settings a visual inspection of these PEF records has proven to be an objective method in identifying individuals with occupational asthma[124,127]. However, in large scale epidemiological studies the visual inspection of PEF records is not practical and the use of a numerical expression of the PEF variability or change in PEF is to be preferred. Of the various proposed indices of PEF variability[128,135-138], the amplitude as percentage of the mean PEF was the measure giving the greatest separation between subject with and without asthma[128]. Yet, in our study initial analyses showed that the amplitude as well as the Δ PEF presented as a percentage of the mean PEF resulted in associations of these PEF indices with age, gender, height and average PEF. This suggested that correction of the indices for mean PEF introduced correlations with other variables. This effect could also be present in other studies in which associations between amplitude percentage mean and gender[136,139], age[140,141] or mean PEF[141] have been found. Therefore, in this study the absolute values in l/min were used in all analyses.

In this study several indices of PEF variability and changes in PEF were used. Of these indices the Δ AMP and Δ PEF describe differences in PEF between days with and without exposure, i.e. contact with laboratory animals. The effect of exposure on the PEF variability has been studied in several occupational studies[129-131]. Two of these studies[130,131] found a relationship between exposure to occupational agents and PEF variability, but these studies did not differentiate between days away from and at work. In our study the amplitude of the PEF was significantly elevated for the workers with chest tightness due to working with rats compared to the workers without symptoms. However, the amplitude was elevated on working days with as well as without contact with laboratory animals (table 2). Therefore, the amplitude can probably be regarded as indicator of non-specific bronchial reactivity[138,140,141] instead of indicator of airway reactivity due to occupational exposure. In contrast, exposure had an effect on the level of the PEF among workers with chest tightness due to

working with rats. These workers showed a work related dip in PEF, i.e. decreased Δ $PEF_{max\text{-}max}$ and Δ $PEF_{min\text{-}min}$ values (table 2). Similarly, Milton and co-workers[132] showed that an index on the basis of change in PEF over the work shift could be used to study effects of exposure in a working population.

A work related decrease in PEF was strongly associated with the presence of chest tightness several hours after working with rats. Interestingly, of all other possible determinants of the Δ $PEF_{min\text{-}min}$, like average level of exposure to RUA, smoking, gender and atopy, only the presence of allergic symptoms to pets was associated with the Δ $PEF_{min\text{-}min}$. Of the workers with allergic symptoms to pets, those who were sensitised to rat allergens as well showed the largest work related decrease in PEF. In previous analyses of data from the same population, we found that allergy to pets was a risk factor of sensitisation to rat allergens[83]. However, not all sensitised workers reported symptoms. It is therefore possible that sensitised workers did not (yet) report chest symptoms due to rat allergens, while at the same time a small work related decrease in PEF could be detected. Therefore, this group of workers is an interesting group to study in the analysis of the follow-up data. At the same time it is relevant to know why a work related dip in PEF is found among workers with allergic symptoms to pets, but not among workers with symptoms to other common allergens. Similarly, a study in a general population[139] showed that sensitisation to cats was more strongly related to bronchial hyperresponsiveness and PEF variability than sensitisation to other common allergens.

On each day the participant had to report working with laboratory animals (yes/no). No information was available on the number of hours working with rats or the actual RUA exposure on each day of measurement. Beside this crude daily measure of exposure, the average level of RUA exposure was used as estimate of the daily exposure. However, this estimate of exposure could not be related to a decrease in PEF. As described elsewhere, there is a high variability of exposure from day to day for each worker[86,100] and this could account for the fact that a relationship between increasing level of average exposure and decrease in PEF was not found. In order to relate changes in PEF with level of exposure to RUA, it is probably necessary to measure the exposure repeatedly as well. This approach was performed in a study among fuel oil ash exposed workers[142].

Several potential biases may have influenced the results. During PEF measurements learning effects can be present[137,141]. In our study these effects were minimised by leaving the first day of the measurement period out of the analysis. Further analyses of the data showed no relation between day of measurement and maximum, minimum and average PEF. Furthermore, workers can falsify their PEF measurements[143-145] or

data errors can occur. Therefore, the graphs of all records were visually inspected. Irregularities were only found during the first day of some of the records, which is in agreement with the possibility of a learning effect. No further irregularities were found in the graphs, probably due to the fact that workers who were not motivated probably failed to return a properly completed PEF record (n = 109) and were therefore not included in the analyses.

Study design may also have influenced the results. Firstly, the PEF variability is reduced when the number of daily measurements is reduced[134,146]. However, reducing the number of daily measurement decreases the sensitivity but has little effect on the specificity in order to distinguish between responder and non-responder[146]. Secondly, the time of measurement is very important. Workers may fail to record the lowest PEF, which is normally present in the morning, when they do not need to get up early, as on weekends or on holiday[147]. Therefore, we only used results on working days in our analyses in order to minimise these problems. Thirdly, the workers had to record their PEF for two weeks, irrespective of the exposure. Due to this study design, 81 of the 289 rat workers had no data on working days with or on working days without exposure to laboratory animals. In addition, of the 208 remaining workers 110 workers had data on less than three days with exposure or without exposure. As a result, these three effects of study design may influence the precision of the estimate of a worker's PEF index, but will not have altered the findings in this paper.

This study shows that serial PEF measurements can be useful in detecting short-term responses to an occupational exposure. However, there was a high variation in level of changes in PEF between workers and only a small part of this variation could be explained by known factors. This possibly limits the role of serial PEF measurements in the assessment of occupational asthma in prevalence studies. However, further study on the usability of serial PEF measurements in occupational epidemiological studies is warranted.

CHAPTER 8

GENERAL DISCUSSION

INTRODUCTION

The study presented in this thesis is one of the largest cross-sectional studies on Laboratory Animal Allergy (LAA). The main aim was to study exposure-response relationships of LAA and their modification by host factors, such as atopy, gender and smoking. Several research questions were formulated (Chapter 1) and the answers to these research questions will be discussed in more detail in this chapter. The findings presented in this thesis may be useful for standard setting and prevention of future occurrence of LAA in exposed workers when the findings are accepted as valid. Therefore, this chapter critically examines the validity of the findings. Additionally, our findings are compared with results reported in the literature. This chapter concludes with suggestions for prevention of LAA and recommendations for further study.

MAIN FINDINGS

LABORATORY ANIMAL ALLERGEN EXPOSURE

The ability to quantitatively measure exposures is a major advantage in epidemiology and risk assessment[122]. We developed two immunoassays to measure Rat (RUA) and Mouse Urinary Allergens (MUA) in airborne dust samples. Both immunoassays appeared to be very sensitive and levels as low as 0.055 ng rat or mouse urinary protein per ml extraction buffer could be measured for stationary total dust samples and 0.075 ng/ml for personal inhalable dust samples. Due to differences in sampling time and flow rate this resulted in detection limits for field samples of 0.030 ng eq/m^3 for ambient air total dust samples (extraction volume 4.5 ml, average sampled volume 9.4 m^3), 0.23 ng eq/m^3 for inhalable dust shift samples (extraction volume 2 ml, average sampled volume 0.64 m^3), and 0.94 ng eq/m^3 for inhalable dust task samples (extraction volume 2 ml, average sampled volume 0.16 m^3, Chapters 2 and 3).

The rabbit polyclonal antibodies used in the immunoassays, reacted to all potential allergens present in rat and mouse urine (figures 1 and 2, Chapter 2). It is, however, possible that two samples with the same level of allergenic reactivity in the assay, might contain different compositions of RUA or MUA. For example, two air samples were taken and the RUA concentration in both samples was 2 ng/m^3. However, one sample contained 80% of *Rat n* IA (urinary protein of 20-21 kd) and 20 % of *Rat n* IB (urinary protein of 16-17 kd), whereas the other sample contained 50% of both proteins. Therefore, we preferred to express the allergen concentrations in ng protein *equivalent* per m^3, in which 1 ng eq was defined as the amount of allergenic activity giving the same OD_{492} as 1 ng protein of the standard. Additionally, the concentrations presented in this study, can not be compared directly with the allergen concentrations

reported in various other studies (Chapter 4). The reported allergen concentrations presented by different researchers depend largely on the extraction method, reference preparation, type of antibodies and type of immunoassay used.
In our study, animal caretakers and animal technicians experienced the highest allergen exposure (Chapter 2 and 3). However, the large variability in exposure within each job title was striking and indicates that information regarding the job performed is not sufficient to be used as a surrogate measure of exposure in epidemiological studies. The zone in which the participant was working explained a larger percentage of the variability in RUA levels than job title. Zone was, therefore, used for grouping the workers (Chapter 6).

DETERMINANTS OF LABORATORY ANIMAL ALLERGEN EXPOSURE

Large differences in ambient air RUA and MUA concentrations were found when various animal rooms were compared (Chapter 3). These samples were taken in 40 rooms of 7 participating facilities. The variability in allergen concentration could be explained for more than half by the number of animals in the room. The RUA and MUA level increased approximately 1.6 and 1.8 times when the number of rats or mice doubled, respectively. Additionally, the activity of the animals had a large effect on the ambient air allergen level in the animal rooms. The inverse day/night rhythm, which makes rats more active during working hours, resulted in 11 times higher RUA levels in the rooms. Chapter 3 also showed that the RUA and MUA concentrations were 6 and 17 times lower in rat and mouse rooms where filter top cages were used, compared to similar rooms without filter top cages.
On Mondays the ambient air RUA and MUA levels were significantly elevated compared to the levels on the other days of the week (Chapter 3). In addition, the personal RUA and MUA exposure levels were also elevated on Mondays. On Mondays, approximately 70 percent of the time is spent on cleaning out dirty cages and changing animals into new cages. These tasks, which could be summarised as working with contaminated bedding material and handling large numbers of conscious animals, were associated with the highest aeroallergen exposure (Chapter 3). Therefore, the proportion of time spent on these tasks will determine the RUA or MUA exposure of the workers to a large extent. However, considerable variability in allergen exposure within each task remained unexplained. Some of this variability may be due to differences between sites. Possible factors are the number of animal present in the facility or the presence of special control measures, like isolators or filter top cages. Other site-specific factors which may contribute to the differences in personal exposure levels, are the level of contamination of the bedding material,

personal behavior of the worker, e.g. pace with which tasks are performed, and type and level of local ventilation or other exposure control equipment. However, these determinants were not investigated in our study.

PREVALENCE RATE OF LABORATORY ANIMAL ALLERGY

Occupational allergy is a major health problem among persons working with laboratory animals in the Netherlands. In our study 18.8% of the rat workers reported allergic symptoms caused by rats (chest tightness (asthma), eye/nose or skin symptoms, Chapter 5). Among mouse workers allergic symptoms caused by mice were reported by 10.1% of the workers. When allergic symptoms caused by other small animals, like rabbits and guinea pigs, are included, 23% of the all workers reported work related allergic symptoms. Nose/eye symptoms were reported most frequently (rats 16.8%, mice 9.0%), followed by skin symptoms (rats 10.7%, mice 4.2%) and asthmatic symptoms (rats 6.1%, mice 3.2%).

Sensitisation to rat allergens, measured by skin prick tests and specific IgE analysis, was present in 18.2% of the rat workers. Sensitisation to mouse allergens was present in 10.7% of the mouse workers. Symptoms, especially asthmatic symptoms, and sensitisation correlated strongly. Of the rat workers with allergic symptoms, 69% were sensitised to rat allergens. For workers with asthmatic symptoms 82% were sensitised. For mouse workers these associations were less pronounced, 39% and 58%, respectively (Chapter 5).

EXPOSURE-RESPONSE RELATIONSHIP

It has been suggested that substantial relative risks can no longer be expected in occupational epidemiological studies[148]. Nevertheless, our study showed that large relative risks can still be found among workers exposed to airborne laboratory animal allergens. Additionally, our study clearly showed that a relationship between level of exposure to rat urinary aeroallergens and prevalence rate of sensitisation to rat allergens exists. The prevalence rates of sensitisation to rat allergens for the atopic workers were about 15, 9.5 and 7.3 times higher in the high, medium and low exposure group, respectively, compared to an internal reference group (Chapter 6).

On each day during a period of two weeks the participants recorded their Peak Expiratory Flow (PEF) and reported working with laboratory animals (yes/no) (Chapter 7). This chapter showed that the PEF of workers who reported asthmatic symptoms decreased significantly on days when they worked with laboratory animals. This effect was more pronounced among workers with a late response, i.e. the presence of asthmatic symptoms several hours after working with rats. In addition, workers with

asthmatic symptoms were also more likely to have a higher PEF variability than workers without asthmatic symptoms, even if workers without asthmatic symptoms experienced skin, eye or nose symptoms due to working with rats.
These exposure-response analyses could not be performed for mouse workers, due to the small population of mouse workers and low prevalence rate of sensitisation to mouse allergens among the mouse workers with less than 4 years of working experience.

MODIFYING FACTORS OF THE EXPOSURE-RESPONSE RELATIONSHIP

Various indices of 'atopy' were used in our study (Chapter 5). All indices were associated with rat and mouse allergy. Interestingly, we found that allergy to cats and dogs, defined by SPT or questionnaire, was highly associated with rat and mouse allergy. In contrast, the prevalence rates of rat and mouse allergy in workers without SPT responses to cat and dog fur but positive to at least one of the other common allergens, i.e. grass pollen, tree pollen, or house dust mite, were comparable with those among workers with no SPT response to any common allergens.
Elevated total IgE (≥ 100 kU/l) was also strongly associated with the prevalence rate of rat and mouse allergy. When three indices of atopy, i.e. 'history of allergic symptoms to pets', 'positive SPT response to cat or dog fur' and 'elevated total IgE', were combined, a very strong association with the prevalence rate of rat and mouse allergy was found (Chapter 5). The prevalence rates of rat and mouse allergy were 1.8% and 0.43%, respectively, when none of the three 'atopy associated risk factors' was present. The prevalence rates of rat and mouse allergy increased with increasing number of these 'atopy associated risk factors' present, from respectively 21% and 6.3% with one 'atopy associated risk factor', to respectively 46% and 17% with at least two 'atopy associated risk factors'.
The association between allergen exposure and sensitisation to rat allergens (Chapter 6) clearly varied between workers with and without 'atopy associated risk factors'. In addition, the exposure related increase in sensitisation was larger in workers with atopy associated risk factors, which suggested a combined effect of exposure and atopy associated risk factors on the development of LAA. Atopy associated risk factors should, therefore, be considered as effect modifiers of exposure. No further distinction between the number of 'atopy associated risk factors' could be made in our exposure-response analyses, due to the relatively small population size of workers with less than four years of working experience with laboratory animals.
After taking into account the exposure level and the atopy associated risk factors, sensitisation to rat allergens was about twice as prevalent in men compared to women,

and about 1.6 times more prevalent among smokers compared to non-smokers (Chapter 6). Both differences were, however, not statistically significant.

VALIDITY

The validity of the study presented in this thesis is a prerequisite for an useful contribution to standard setting and prevention of future occurrence of LAA in exposed workers. Firstly, the internal validity will be discussed. In general, three types of biases can detract from internal validity, i.e. selection bias, information bias and confounding[149]. Secondly, the validity of the results to other laboratory animal populations will be discussed (external validity).

SELECTION BIAS & PREVALENCE RATE OF LAA

The procedure used to select the study population may have affected the prevalence rate LAA reported in this thesis. In our study, all employees of the participating facilities who work with small laboratory animals or have contact with material from these animals were invited to participate. Of approximately 750 eligible subjects, 579 (77%) participated. Important reasons for not participating were a lack of interest in the study, fear of disclosure of animal related symptoms to the employer, not working with laboratory animals anymore, or being absent during the study period due to illness or being abroad. Unfortunately, no detailed information was available of the workers who did not participate in the study. As a result of the non-response, the presented prevalence rate of LAA could be higher as well as lower than the true prevalence rate.

Another form of selection bias which may have affected the prevalence rate of LAA presented in this thesis, is self-selection bias. Workers with allergic symptoms worked significantly fewer years with laboratory animals (Chapter 5). Selection related to duration of exposure may have reduced the prevalence rate of LAA. This was clearly visible in chapter 6. The prevalence rate of allergic symptoms due to working with rats increased from 19.6% to 23.7% after restricting the population to workers with less than four years of working experience with laboratory animals. In addition, it is possible that contact with laboratory animals during training of subjects may have caused LAA. These subjects with LAA may have decided to change education or profession even before becoming employed as laboratory animal worker. Besides self-selection, pre-employment screening to exclude atopic applicants may also have reduced the prevalence rates of LAA in some of the facilities[150-153]. However, to our knowledge in none of the facilities under study, explicit pre-employment screening strategies were applied to exclude atopic applicants. In conclusion, due to self-

selection bias the prevalence rate of LAA may have been underestimated.

SELECTION BIAS & EXPOSURE-RESPONSE RELATIONSHIP OF LAA

Selection may bias exposure-response relationships, if associated with exposure level. Chapter 6 (table 2) showed that sensitisation to, and symptoms due to rat allergens were less prevalent among highly exposed workers compared to the medium exposed workers. It is possible that highly exposed workers with LAA had left the workforce or were transferred to zones with less exposure, i.e. a 'healthy worker effect'. In our study we were able to minimise bias related to duration of exposure by restricting the population, i.e. selecting the workers with less than four years of working experience with laboratory animals (Chapter 6). Four years was chosen, because most laboratory animal workers were PhD-students with a contract period of four years and one other study on LAA[22] also restricted their population to 'newly' exposed workers, i.e. four years of exposure or less.

An additional point of interest is our reference group. In the exposure-response analyses presented (Chapter 6), the reference group consisted of 86 participants, who had never worked with rats. The workers of this group perform similar tasks as the rat workers, although, they use different animals or material derived from different animals. In our study these participants worked predominantly with mice, but to a certain extent also with rabbits, guinea pigs, cats or dogs. However, the workers of the internal reference group will also be exposed to rat allergens when working in the animal facilities. In some of the facilities the rooms in which the different species of animals were housed, were in the vicinity of each other. As a result 3 of the 86 workers of the references group were sensitised to rat allergens. The internal reference group, therefore, should not be regarded as a completely 'non-exposed' group, but is still the best reference group possible, when studying monocausal diseases like rat allergy. This internal reference group may have diminished the observed prevalence rate ratios, but will not have influenced the exposure-response relationship present among the rat workers (Chapter 6). Despite the limitations using the internal reference group, the prevalence rate ratios remained large.

INFORMATION BIAS & PREVALENCE RATE OF LAA

Bias can occur from errors in obtaining information on either disease or exposure[149]. In our study, LAA was assessed by subjective, i.e. reported allergic symptoms, as well as by objective means, i.e. sensitisation by skin prick and specific IgE test. At this moment, no validated questionnaire for evaluating occupational allergy exists[26]. Our questionnaire was based on a questionnaire used in a British study on LAA[22]. In

contrast to standardised respiratory questionnaires previously used in studies on LAA, like the American Thoracic Society adult questionnaire (ATS-DLD) or the British Medical Research Council (MRC) questionnaire, the British questionnaire[22] did not only include questions on asthma, but also on allergic symptoms. Questions were asked on chest tightness (asthma), runny or sneezing nose, runny or itchy eyes, and itchy or red skin (Chapter 5). Additionally, questions were added on the timing of the allergic symptoms, i.e. during or several hours after contact to laboratory animals, and type of animal causing the symptoms.

In epidemiological studies on occupational allergy it is important to ensure that reported allergic symptoms are indeed provoked by the allergen under study. In recently published guidelines for epidemiological assessment of occupational asthma[43], it has been suggested that occupational asthma or in general occupational allergy should not be assessed solely by the prevalence of symptoms using a questionnaire, which is a sensitive but non-specific tool[44], but should be used in combination with results of SPT or specific IgE tests, or serial PEF measurements.

In Chapter 5 it was shown that 69% of the 86 rat workers with allergic symptoms were sensitised to rat allergens. For workers with asthmatic symptoms (28 of the 86 rat worker with allergic symptoms), 82% were sensitised. For the 38 mouse workers with allergic symptoms, these associations were less pronounced, 39% and 58%, respectively. It is possible that the skin prick test and the specific IgE measurement were not sensitive enough. However, changing the cut-off level of a positive IgE response from 0.70 kU/l (Class 2) to 0.35 kU/l (Class 1), or of a positive SPT response to occupational allergens from 4 mm to 3 mm, hardly increased the number of symptomatic workers who were also sensitised. However, these changes in cut-off points clearly increased the number of sensitised workers who did not report symptoms. A more plausible explanation of the presence of symptoms in absence of sensitisation, is that not all symptoms observed were IgE-mediated. The symptomatic workers who were not sensitised, had an elevated prevalence rate of positive SPT responses to house dust mites, and of self-reported hyperresponsiveness compared to the workers without symptoms who were also not sensitised (Chapter 5). Therefore, the symptoms of these symptomatic workers after contact with rats or mice may result from a non-specific hyperresponsiveness to animal-derived or other agents, such as dust, disinfectants or ammonia, which are present simultaneously.

In Chapter 7 it was shown that the PEF of workers who reported asthmatic symptoms due to working with rats was significantly decreased on days when contact with laboratory animals was present. A decrease in PEF due to exposure to laboratory animal allergens was not observed in workers without asthmatic symptoms, not even

in the subgroup of workers without asthmatic symptoms who experienced skin, eye or nasal symptoms.

In conclusion, in order to distinguish between different causes of reported allergic symptoms we assumed that 'rat allergy' or 'mouse allergy' was most likely present if subjects reported allergic symptoms due to working with rats or mice and were also sensitised to rat or mouse allergens. However, this stringent definition of rat and mouse allergy may have underestimated the true prevalence slightly.

INFORMATION BIAS & EXPOSURE-RESPONSE RELATIONSHIP OF LAA

Errors in the classification of subjects with and without LAA may also have biased the exposure-response relationships presented in Chapter 6. During the medical testing of the workers, the workers as well as the medical technicians were unaware of the worker's actual exposure, i.e. their 'time-multiplied exposure'. Therefore, misclassification of subjects due to our strategy will be independent of exposure and therefore nondifferential. In general, nondifferential misclassification will bias the effect estimate toward the null value and may mask the true risk. It could, therefore, not account for the observed exposure-response relationships.

In general, nondifferential misclassification of exposure will bias the effect estimate toward the null value and may mask the true risk. However, for a 'grouping strategy' this is different. It has been suggested that whatever the grouping will be, the relationship between exposure response is unbiased[85,154]. This has been described as the 'Berkson-type error'. In our study the rat workers were initially grouped on the basis of zone working in. However, grouping the workers on the basis of zone did not account for the actual time working with rats. Therefore, the hours working with rats per week, which has been determined by questionnaire, was taken into account. This 'time-multiplied RUA exposure' was used for grouping the workers. According to the 'Berkson-type error', the slope of the exposure-response relationship (Chapter 6) was not dependent on arbitrary decisions made in applying this 'time-multiplied RUA exposure' for grouping the workers.

CONFOUNDING BIAS

A confounding variable must be a risk factor for the disease, associated with the exposure under study and not an intermediate step in the causal path between exposure and disease[149]. In the literature exposure, atopy, gender, smoking and age have been suggested as risk factors of LAA[155].

General population studies did find sensitisation to common allergens to be more prevalent in men[73-77]. In agreement with this observation our study showed atopy

associated risk factors to be more prevalent in male than female rat workers, 38.5% to 30.8%, respectively. This higher prevalence rate of atopy associated risk factors, the higher 'time-multiplied RUA exposure' levels for men, 9.0 hours weekly ng eq/m^3 versus 4.6 hours weekly ng eq/m^3 for women and the higher prevalence rate of sensitisation to rat allergens in men (Chapter 6), makes gender a potential confounder. In addition, associations were found between smoking and exposure, atopy and rat allergy (Chapter 6). Therefore, the confounding effect of gender and smoking was taken into account in the analyses presented in Chapter 6. However, the crude prevalence rate ratios changed only slightly after adjusting for gender and smoking. In our study, we had no data on the smoking habits at the time of first exposure. It is possible that smoking habits were influenced by the development of symptoms or that subjects allergic to common allergens would less easily pick up the habit of smoking, which may result in some residual confounding.

In our study, age was associated with rat allergy in the total group of rat workers. However, for the exposure-response analyses we restricted our population to subjects who were working with laboratory animal for less than 4 year. Therefore, the association between age and rat allergy was no longer present and not a potential confounder. The presence of pets was not a potential confounder, because no association with LAA was observed.

EXTERNAL VALIDITY

Our study clearly shows that a relationship between exposure to rat urinary aeroallergens and prevalence rate of rat allergy. However, the slope of the exposure-response relationship may be influenced by our study design. In our study the workers with less than four years of working experience with laboratory animals were used in the exposure-response analyses (Chapter 6). The cut-off point of four years is arbitrary, but sensitivity analyses with a restricted population using 3 or 5 years as cut-off points gave similar results. However, 4 years may be too short to become sensitised or to develop symptoms in the low exposure categories or if workers are not 'atopic'. A recently performed retrospective cohort study in the Netherlands[37] showed a median time until development of allergic symptoms due to working with laboratory animals of approximately 98 months in non-atopic and 27 months in atopic workers. The median years of exposure was 2 years in our restricted population. In addition, the time until development of symptoms has been suggested to be shorter in highly exposed workers[37]. More conclusive answers can only be given after analysis of follow-up data.

RECENT FINDINGS IN THE LITERATURE

In our study the overall prevalence rate of allergic symptoms due to working with rats (asthmatic, eye/nose or skin symptoms) was 19% (Chapter 5), which is roughly similar to the prevalence rates found in other studies on rat allergy in the US (12%[14]), UK (18%[17], 31%[22]) and Japan (25%[20]). The prevalence rate of mouse related symptoms was 10% and tended to be lower than described in most other studies (UK 11%[17]; Japan 26%[20]; Australia 32%[11]). As mentioned before in this chapter, the comparison with other studies should be made with care, due to differences in methods measuring rat and mouse allergy and differences in population characteristics.

In addition to our study, two other studies described a relationship between exposure to rat urinary aeroallergens and rat allergy[18,22]. In one of these studies three exposure categories were distinguished, based on a large number of full-shift personal air measurements[22]. Allergic skin symptoms were significantly more present at medium (prevalence rate of 12%) and high RUA exposure levels (prevalence rate of 17%) compared to the category with low exposure levels (prevalence rate of 3%). However, no relationship was observed for chest, eye, and nasal symptoms, and sensitisation to rat allergen. In the other study[18], the estimated task-specific rat antigen concentration was multiplied by the duration of performed tasks. This measure of exposure was positively associated with the prevalence rate of LAA symptoms. However, detailed information on the exposure levels used in the analyses was not available and a potential modifying effect of host factors on the exposure-response relationship was not taken into account. Furthermore, no distinction was made between species of animals causing the symptoms.

The existence of exposure-response relationships between level of exposure and sensitisation to aeroallergens has also been suggested in a limited number of studies on other high molecular aeroallergens. In occupational studies more workers were sensitised to α-amylase [29] or *Bacillus subtilis* enzymes [121] in groups with high exposure. In these populations the exposure-response relationships were also more pronounced in atopic workers.

Atopy has also been found to be a strong risk factor of LAA in other studies[8,11,12,14,17,19,21,34,35,69-71]. We found that allergy to cats and dogs was highly associated with rat and mouse allergy. This was also found in two other studies on LAA. In a study among fifty-six laboratory animal workers[21] a positive SPT response to common allergens was associated with LAA (OR = 9.3, 95% CI 2.4 - 37), but when the response to dog and horse was excluded, the association was much weaker (OR = 3.3, 95% CI 0.7 - 16). In another study[70] five out of ten workers with LAA were SPT

positive to dog and/or horse and all symptom free workers were SPT negative to these animals. However, due to the cross-sectional analyses in our study as well as in the other two studies[21,70], it was not possible to verify if the SPT response to pets and an elevated total IgE precede LAA. More conclusive answers can only be given after analysis of follow-up data. A recently performed study[156] suggested that a positive SPT response to dog fur/dander allergens, may be due to contamination of the extract with house dust mite. However, our extract was prepared by Diephuis (ALK Benelux, Groningen), and the *Der p* 1 level found in this extract was too low to cause false positive reactions[156]. Therefore, false positive SPT response to dog fur due to contamination by *Der p* 1 seems not present in our study. In addition, 46% of the workers with a positive SPT response to dog fur had a negative response to house dust mite in our study.

Elevated total IgE (≥ 100 kU/l) was also strongly associated with rat and mouse allergy, which is in agreement with other studies[19,34,70]. In the follow-up study of Renström et al.[34], 8 of the 9 workers with LAA had an elevated total IgE level prior to exposure, compared to only 8 of the 29 non-LAA subjects. Therefore, 7 out of 15 (47%) subjects with an elevated total IgE prior to exposure, had developed LAA. In contrast, only 1 out of 23 (4%) subjects without an elevated total IgE, had developed LAA. This study shows with a relative risk of approximately 11, that elevated total IgE is indeed a risk factor of LAA

In previous studies on occupational allergy, the effect of gender received only limited attention. No more than two studies have previously considered the association between gender and LAA[14,72]. No differences in prevalence of LAA have been found between men and women in these reports. However, in our study, after taken level of exposure, atopy associated risk factors and smoking into account, sensitisation to rat allergens was still about twice as prevalent in men (Chapter 6). This difference was not statistically significant due to the small sample size, but it suggests that gender is an independent risk factor of LAA.

In our study sensitisation to rat allergens was about 1.6 times more prevalent among smokers. However, this finding was not statistically significant. There is conflicting evidence regarding the role of smoking in sensitisation to occupational allergens. Some studies[22,71,72] have found sensitisation to laboratory animals allergens to be more common in smokers, while several other studies[10,12,14,15] failed to demonstrate this relationship. An explanatory factor might be the cross-sectional design of most studies. It is possible that smoking habits were influenced by the development of symptoms or that subjects allergic to common allergens would less easily pick up the habit of smoking.

PREVENTION

Reduction of exposure to laboratory animal allergens is the most direct way of preventing LAA. In the Netherlands employers have the obligation to adapt the workplace to the most sensitive worker, who should be able to work in a normal situation with a low risk of developing occupational allergy. In Chapter 6 the relationship between exposure to RUA and the prevalence rate of rat allergy was described. This relationship was more pronounced in 'atopic' workers. However, even at low 'time-multiplied exposure' levels (< 1 hours weekly ng eq/m^3) 27% of the 'atopic' workers were sensitised to rat allergens and 9% of these workers had a rat allergy, i.e. were sensitised and reported symptoms. In contrast, among the 'non-atopic' workers, non of the workers was sensitised to rat allergens in the lowest exposed group. On the basis of the measurements presented in Chapter 3, this 'time-multiplied exposure' level (1 hours weekly ng eq/m^3) could be reached by changing rats for about nine minutes per week or being in an 'average rat room' for slightly more than one hour per week. Consequently, large reductions in exposure level are necessary for this group of sensitive workers, i.e. 'atopic' workers, to be able to work under normal conditions with a low risk of developing occupational allergy.

Exposure to rat and mouse aeroallergens can be controlled in various ways, of which the most essential measures are: reduction of the emission of the allergens, isolation of animals, ventilation, avoidance of exposure and personal protection[85,157]. In the Netherlands, the applied control measures have, in general, been focused on reduction of background concentrations of airborne allergens in animal rooms. Of these control measures, an increase in ventilation rate or optimisation of the ventilation in animal rooms is most often applied. However, a substantial increase in ventilation rate is necessary to achieve a substantial reduction[96]. A high ventilation rate will, however, cause draught, which is uncomfortable to work in and may also have a negative influence on the health of the animals. A better alternative for reduction of background concentrations of airborne allergens in animal rooms is the use of filter top cages. The use of filter top cages was shown to give a large reduction of the ambient air allergen concentration (Chapter 3)[68]. However, in the Netherlands filter top cages have only been used to isolate infected animals or to prevent animals from getting infected. A very promising way for reduction of background concentrations of airborne allergens in the animal rooms is the use of plastic curtains in front of the animal cages[157,158]. These curtains reduce the allergen concentration in the centre of the animal room substantially.

Measures to reduce the ambient air allergen level in animal rooms may result in low allergen exposure of the workers when entering a room for inspection of animals or

when performing tasks that do not disturb the animals. However, our study showed that the highest exposures were observed during the handling of animals or during contact with contaminated bedding material (Chapter 3). Reduction of animal allergen exposure during these highly exposed tasks should receive more attention in the Dutch animal facilities. Possible measure for reduction of allergen exposure during these highly exposed tasks are:

- choosing appropriate bedding material. Woodchips or sawdust, which are normally used as bedding material, can be very dusty when being removed from the cages (cleaning out of cages), during the handling of animals and when the animals are disturbed. For instance, the use of absorbent pads as bedding material may reduce the amount of allergens getting airborne[68,94]. Furthermore, absorbent pads resulted in a 90% reduction of the exposure during the cleaning out of cages when compared to cages containing woodchips or sawdust[68].
- if woodchips or sawdust are used, special vacuum cleaners or ventilation benches should be used when cleaning out the cages. These vacuum cleaners may also be used for cleaning animal rooms.
- the handling of animals as well as biotechnical and experimental work on the animals should be performed on special ventilated benches or in ventilated cabinets[157-160].
- allergen exposure should be avoided. Activities that involve the use of laboratory animals should be separated from the rest of the research facility, for example by dividing the facility into an 'animal' and 'animal free' part. No animals or high contaminated bedding material should be allowed to be present outside the 'animal part' of the facility. Animals or bedding material may only be transported in special 'transporting cages'. These cages should by equipped with special filters to minimise the exposure.
- personal protection can be used as final piece of a control strategy in which the measure mentioned above can not be applied. Personal protection, if used properly, may achieve in these situations an appropriate reduction of exposure[161-163].

Finally, motivation by the workers and good organisation by the laboratory animal facility are essential prerequisites for the prevention of LAA. Furthermore, all suggestions for reduction of the allergen exposure are based on the results presented in this thesis and in other studies, and on personal impressions obtained during the time spent in the laboratory animal facilities to measure the exposure to laboratory animal allergens. However, more work is needed to determine the effectiveness of control measures in reducing the incidence of LAA. We should realise that such measures

may conflict with the health of the animals.

'Atopic status' has been found to be a strong risk factor of LAA (Chapter 5)[8,11,12,14,17,19,21,34,35,69-71]. It has been suggested that reduction of LAA can be achieved by pre-employment screening on 'atopic status'[19,164]. However, the use of 'atopy status' does not meet some of the criteria which have been proposed for pre-employment screening[165]. Firstly, in the literature several different tests, e.g. skin prick test or total IgE measurement, and different criteria, e.g. differences in common allergens used or differences in cut-off level of total IgE, have been used to determine 'atopic status'. However, these various indices can not be used synonymously in studies on LAA (Chapter 5)[19,69,70,152]. Additionally, very little is known about the sensitivity, specificity, and predictive value of the various tests and criteria. Secondly, 'atopic status' is highly prevalent among laboratory animal workers and only a proportion of these 'atopic' workers would develop LAA. For example, in our study 39% of the workers had at least one positive response to the 'atopy associated risk factors' (Chapter 5). In this group of workers approximately one-third reported symptoms of allergy caused by rats or mice. Therefore, two-third reported no symptoms of allergy caused by rats or mice and would have been denied employment if pre-employment screening would have been applied. Additionally, approximately 7% of the workers without 'atopy associated risk factors' reported symptoms of allergy caused by rats or mice.

In conclusion, there is insufficient data available to use pre-employment screening on 'atopic status' as an 'acceptable' method of preventing LAA at this moment. The emphasis should be on exposure reduction, which is the most direct way of reducing LAA. However, the 'atopic status' of a worker may be determined in order to give individuals an informed view on their risk of developing LAA.

CONCLUSIONS AND SUGGESTIONS FOR FURTHER STUDY

The study presented in this thesis showed that Dutch laboratory animal workers were highly exposed when performing tasks which involve contaminated bedding material or large number of animals. However, large differences were observed between the various laboratory animal facilities. In this study we found a relationship between animal allergen exposure level and the prevalence rate of LAA. In addition, atopy was a major risk factor of LAA, as well as an effect modifier of laboratory animal exposure.

Suggestions for further study are:

- longitudinal data need to be collected to verify if exposure and atopy are indeed

risks factors of LAA.

- the role of smoking requires further study in a longitudinal design.
- the effect of duration of exposure on the development of sensitisation and symptoms to animal allergens needs further study.
- for further epidemiological studies and standard setting, standardisation of methods to measure airborne allergen exposure is needed.

REFERENCES

1. Malo JL, Chan-Yeung M. Evaluation of impairment/disability in subjects. In: Bernstein L, Chan-Yeung M, Malo JL, Bernstein DI, eds. Asthma in the workplace. New York, Basel, Hong Kong: Marcel Dekker,Inc. 1993:341-358.
2. Grammer LC, Patterson R. Immunologic evaluation of occupational asthma. In: Bernstein L, Chan-Yeung M, Malo JL, Bernstein DI, eds. Asthma in the workplace. New york, Basel, Hong Kong: Marcel Dekker, Inc. 1993:125-143.
3. Chan-Yeung M, Malo JL. Aetiological agents in occupational asthma. Eur Respir J 1994;7:346-371.
4. Meredith SK, McDonald JC. Work-related respiratory disease in the United Kingdom, 1989-1992: report on the SWORD project. Occup Med 1994;44:183-189.
5. Sallie BA, Ross DJ, Meredith SK, McDonald JC. SWORD'93. Surveillance of work-related and occupational disease in the UK. Occup Med 1994;44:177-182.
6. Ross DJ, Sallie BA, McDonald JC. SWORD '94: surveillance of work-related and occupational respiratory disease in the UK. Occup Med 1995;45:175-178.
7. Matte TD, Hoffman RE, Rosenman KD, Stanbury M. Surveillance of occupational asthma under the SENSOR model. Chest 1990;98 supplement:173S-178S.
8. Gross NJ. Allergy to laboratory animals: epidemiologic, clinical, physiologic aspects, and a trial of cromolyn in its management. J Allergy Clin Immunol 1980;22:158-165.
9. Davies GE, McArdle LA. Allergy to laboratory animals: a survey by questionnaire. Int Archs Allergy appl Immun 1981;64:302-307.
10. Slovak AJM, Hill RN. Laboratory animal allergy: a clinical survey of an exposed population. Br J Ind Med 1981;38:38-41.
11. Schumacher MJ, Tait BD, Holmes MC. Allergy to murine antigens in a biological research institute. J Allergy Clin Immunol 1981;68:310-318.
12. Cockcroft A, Edwards J, McCarthy P, Andersson N. Allergy in laboratory animal workers. Lancet 1981;827-830.
13. Beeson MF, Dewdney JM, Edwards RG, Lee D, Orr RG. Prevalence and diagnosis of laboratory animal allergy. Clin Allergy 1983;13:433-442.
14. Bland SM, Levine MS, Wilson PD, Fox NL, Rivera JC. Occupational allergy to laboratory animals: an epidemiological study. J Occup Med 1986;28:1151-1157.
15. Agrup G, Belin L, Sjöstedt L, Skerfving S. Allergy to laboratory animals in laboratory technicians and animal keepers. Br J Ind Med 1986;43:192-198.
16. Botham PA, Davies GE, Teasdale EL. Allergy to laboratory animals: a prospective study of its incidence and of the influence of atopy on its development. Br J Ind Med 1987;44:627-632.
17. Venables KM, Tee RD, Hawkins ER, Gordon DJ, Wale CJ, Farrer MN, Lam TH, Baxter PJ, Newman Taylor AJ. Laboratory animal allergy in a pharmaceutical company. Br J Ind Med 1988;45:660-666.
18. Kibby T, Powell G, Cromer J. Allergy to laboratory animals : a prospective and cross-sectional study. J Occup Med 1989;31:842-846.
19. Sjöstedt L, Willers S. Predisposing factors in laboratory animal allergy: a study of atopy and environmental factors. Am J Ind Med 1989;16:199-208.

20. Aoyama K, Ueda A, Manda F, Matsushita T, Ueda T, Yamauchi C. Allergy to laboratory animals: an epidemiological study. Br J Ind Med 1992;49:41-47.
21. Willers S, Hjortsberg U, Wihl JA, Orbæk P. Sensitization to laboratory animals and small-airway hyperreactivity. Indoor Environ 1992;1:82-87.
22. Cullinan P, Lowson D, Nieuwenhuijsen MJ, Gordon S, Tee RD, Venables KM, McDonald JC, Newman Taylor AJ. Work related symptoms, sensitisation, and estimated exposure in workers not previously exposed to laboratory rats. Occup Environ Med 1994;51:589-592.
23. Health & Safety Executive. Medical aspects of occupational asthma. Guidance note MS 25 from the Health and Safety Executive. HMSO, 1991.
24. Becklake MR. Epidemiology: prevalence and determinants. In: Bernstein L, Chan-Yeung M, Malo JL, Bernstein DI, eds. Asthma in the workplace. New York, Basel, Hong Kong: Marcel Dekker,Inc. 1993:29-59.
25. Malo JL, Chan-Yeung M. Population surveys of occupational asthma. In: Bernstein L, Chan-Yeung M, Malo JL, Bernstein DI, eds. Asthma in the workplace. New York, Basel, Hong Kong: Marcel Dekker, Inc. 1993:145-170.
26. Chan-Yeung M. Occupational asthma. Chest 1990;98 supplement:148S-161S.
27. Becklake MR. Epidemiology and surveillance. Chest 1990;98 supplement:165S-172S.
28. Venables KM. Epidemiology and the prevention of occupational asthma. Br J Ind Med 1987;44:73-75.
29. Houba R, Heederik D, Doekes G, van Run P. Exposure-sensitization relationship for a-amylase allergens in the baking industry. Am J Respir Crit Care Med 1996;154:130-136.
30. Sorrell AH, Gottesman J. Mouse allergy - a case report. Ann Allergy 1957;15:662-663.
31. Rajka G. Ten cases of occupational hypersensitivity to laboratory animals. Acta Allergologica 1961;16:168-176.
32. Lincoln TA, Bolton NE, Garrrett AS. Occupational allergy to animal dander and sera. J Occup Med 1974;16:465-469.
33. Davies GE, Thompson AV, Niewola Z, Burrows GE, Teasdale EL, Bird DJ, Phillips DA. Allergy to laboratory animals: a retrospective and a prospective study. Br J Ind Med 1983;40:442-449.
34. Renström A, Malmberg P, Larsson K, Sundblad B, Larsson PH. Prospective study of laboratory-animal allergy: factors predisposing to sensitization and development of allergic symptoms. Allergy 1994;49:548-552.
35. Botham PA, Lamb CT, Teasdale EL, Bonner SM, Tomenson JA. Allergy to laboratory animals: a follow-up study of its incidence and of the influence of atopy and pre-existing sensitisation on its development. Occup Environ Med 1995;52:129-133.
36. Heederik D, Kruize H, Martens B. Laboratory animal allergy (LAA): a retrospective cohort study using pre-employment data. Eur Respir J 1995;8, supplement 19:193s
37. Kruize H, Post W, Heederik D, Martens B, Hollander A, van der Beek E. Respiratory allergy in laboratory animal workers: a retrospective cohort study using pre-employment screening data (submitted).
38. Patterson R, Grammer LC, Zeiss CR, Harris KE, Shaugnessy MA. Use of immunologic technology in the diagnosis of environmental and occupational immunologic lung disease. Chest 1990;98, supplement:206s-208s.
39. Watt AD, McSharry CP. Laboratory animal allergy: anaphylaxis from a needle injury. Occup Environ Med 1996;53:573-574.
40. Eggleston PA, Ansari AA, Ziemann B, Adkinson NF, Corn M. Occupational challenge studies with laboratory workers allergic to rats. J Allergy Clin Immunol 1990;86:63-72.

41. Das R, Tager IB, Gamsky T, Schenker MB, Royce S, Balmes JR. Atopy and airways reactivity in animal health technicians. A pilot study. J Occup Med 1992;34:53-60.
42. Carroll KB, Pepys J, Longbottom JL, Hughes DTD, Benson HG. Extrinsic allergic alveolitis due to rat serum proteins. Clin Allergy 1975;5:443-456.
43. Smith AB, Castellan RM, Lewis D, Matte T. Guidelines for the epidemiologic assessment of occupational asthma. J Allergy Clin Immunol 1989;84:794-803.
44. Malo JL, Cartier A, L'Archevêque J, Ghezzo H, Lagier F, Trudeau C, Dolovich J. Prevalence of occupational asthma and immunologic sensitization to psyllium among health personnel in chronic care hospitals. Am Rev Respir Dis 1990;142:1359-1366.
45. Newman Taylor A, Longbottom JL, Pepys J. Respiratory allergy to urine proteins of rats and mice. Lancet 1977;847-849.
46. Schumacher MJ. Characterization of allergens from urine and pelts of laboratory mice. Mol Immunol 1980;17:1087-1095.
47. Walls AF, Longbottom JL. Comparison of rat fur, urine, saliva, and other rat allergen extracts by skin testing,RAST, and RAST inhibition. J Allergy Clin Immunol 1985;75:242-251.
48. Lutsky I, Fink JN, Kidd J, Dahlberg MJE, Yuninger JW. Allergenic properties of rat urine and pelt extracts. J Allergy Clin Immunol 1985;75:279-284.
49. Gordon S, Tee RD, Newman Taylor AJ. Analysis of rat urine proteins and allergens by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting. J Allergy Clin Immunol 1993;92:298-305.
50. Longbottom JL. Purification and characterization of allergens from the urines of mice and rats. Advances allergy clin immunol 1979;483-490.
51. Walls AF, Longbottom JL. Quantitative immunoelectrophoretic analysis of rat allergen extracts. II. Fur, urine and saliva studies by crossed radio-immunoelectrophoresis. Allergy 1983;38:501-512.
52. Wahn U, Peters T, Siraganian RP. Studies on the allergenic significance and structure of rat serum albumin. J Immunol 1980;125:2544-2549.
53. Lorusso JR, Moffat S, Ohman JL. Immunologic and biochemical properties of the major mouse urinary allergen (Mus m I). J Allergy Clin Immunol 1986;78:928-937.
54. Price JA, Longbottom JL. Allergy to mice. I. Identification of two major mouse allergens (Ag 1 and Ag 3) and investigation of their possible origin. Clin Allergy 1987;17:43-53.
55. Price JA, Longbottom JL. Allergy to mice. II. Further characterization of two major mouse allergens (AG1 and AG3) and immunohistochemical investigations of their sources. Clin Exp Allergy 1990;20:71-77.
56. Edwards RG, Beeson MF, Dewdney JM. Laboratory animal allergy: the measurement of airborne urinary allergens and the effects of different environmental conditions. Lab Animals 1983;17:235-239.
57. Twiggs JT, Agarwal MK, Dahlberg MJE, Yunginger JW. Immunochemical measurement of airborne mouse allergens in a laboratory animal facility. J Allergy Immunol 1982;69:522-526.
58. Davies GE, Thompson AV, Rackham M. Estimation of airborne rat-derived antigens by ELISA. J Immunoassay 1983;4:113-126.
59. Corn M, Koegel A, Hall T, Scott A, Newill C, Evans R. Characteristics of airborne particles associated with animal allergy in laboratory workers. Ann Occup Hyg 1988;32 supplement 1:435-446.

60. Lewis DM, Bledsoe TA, Dement JM. Laboratory animal allergies. Use of the radioallergosorbent test inhibition assay to monitor airborne allergen levels. Scand J Work Environ Health 1988;14 supplement 1:74-76.
61. Price JA, Longbottom JL. ELISA method for measurement of airborne levels of major laboratory animal allergens. Clin Allergy 1988;18:95-107.
62. Eggleston PA, Newill CA, Ansari AA, Pustelnik A, Lou S, Evans III R, Marsh DG, Longbottom JL, Corn M. Task-related variation in airborne concentrations of laboratory animal allergens : Studies with RAT n I. J Allergy Clin Immunol 1989;84:347-352.
63. Gordon S, Tee RD, Nieuwenhuijsen MJ, Lowson D, Harris J, Newman Taylor AJ. Measurement of airborne rat urinary allergen in an epidemiological study. Clin Exp Allergy 1994;24:1070-1077.
64. Renström A, Gordon S, Larsson PH, Tee RD, Newman Taylor AJ, Malmberg P. Comparison of a monoclonal ELISA and a RAST inhibition method for aeroallergen measurement. Annual meeting EAACI Stockholm,Sweden 1994;456.
65. Gordon S, Kiernan LA, Nieuwenhuijsen MJ, Cook AD, Tee RD, Newman Taylor AJ. Measurement op exposure to mouse urinary proteins in an epidemiological study. Occup Environ Med 1997;54:135-140.
66. Gordon S, Tee RD, Lowson D, Newman Taylor AJ. Comparison and optimization of filter elution methods for the measurement of airborne allergen. Ann Occup Hyg 1992;36:575-587.
67. Nieuwenhuijsen MJ, Gordon S, Tee RD, Venables KM, McDonald JC, Newman Taylor AJ. Exposure to dust and rat urinary aeroallergens in research establishments. Occup Environ Med 1994;51:593-596.
68. Gordon S, Tee RD, Lowson D, Wallace J, Newman Taylor AJ. Reduction of airborne allergenic urinary proteins from laboratory rats. Br J Ind Med 1992;49:416-422.
69. Slovak AJM, Hill RN. Does atopy have any predictive value for laboratory animal allergy? A comparison of different concepts of atopy. Br J Ind Med 1987;44:129-132.
70. Sjöstedt L, Willers S, Orbæk P. A follow-up study of laboratory animal exposed workers: the influence of atopy for the development of occupational asthma. Am J Ind Med 1993;24:459-469.
71. Venables KM, Upton JL, Hawkins ER, Tee RT, Longbottom JL, Newman Taylor AJ. Smoking, atopy, and laboratory animal allergy. Br J Ind Med 1988;45:667-671.
72. Fuortes JL, Weih L, Jones ML, Burmeister LF, Thorne PS, Pollen S, Merchant JA. Epidemiologic assessment of laboratory animal allergy among university employees. Am J Ind Med 1996;29:67-74.
73. Sears MR, Burrows B, Flannery EM, Herbison GP, Holdaway MD. Atopy in childhood. I. Gender and allergen related risks for development of hay fever and asthma. Clin Exp Allergy 1993;23:941-948.
74. Omenaas E, Bakke P, Elsayed S, Hanoa R, Gulsvik A. Total and specific serum IgE levels in adults: relationship to sex, age and environmental factors. Clin Exp Allergy 1994;24:530-539.
75. Nielsen NH, Svendsen UG, Madsen F, Dirksen A. Allergen skin test reactivity in an unselected Danish population. Allergy 1994;49:86-91.
76. Jarvis D, Luczynska C, Chinn S, Burney S. The association of age, gender and smoking with total IgE and specific IgE. Clin Exp Allergy 1995;25:1083-1091.

77. Mensinga TT, Schouten JP, Rijcken B, Weiss ST, Speizer FE, van der Lende R. The relationship of eosinophilia and positive skin test reactivity to respiratory symptoms prevalence in a community-based population study. J Allergy Clin Immunol 1990;86:99-107.
78. Medical Research Council Committee on the aetiology of chronic bronchitis. Instructions for the use of the questionnaire on respiratory symptoms. Dawlish, UK: Holman Ltd, 1966:
79. Heederik D, Brouwer R, Biersteker K, Boleij JSM. Relationship of airborne endotoxin and bacteria levels in pig farms with the lung function and respiratory symptoms of farmers. Int Arch Occup Environ Health 1991;62:595-601.
80. Veterinaire Hoofdinspectie van de Volksgezondheid. Zo doende 1990, jaaroverzicht van de sectie dierproeven. Den haag: WVC, 1991.
81. Zock J, Hollander A, Doekes G, Heederik D. The influence of different filter elution methods on the measurement of airborne potato antigens. Am Ind Hyg Assoc J 1996;57:567-570.
82. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC. Measurement of protein using bicinochoninic acid. Anal Biochem 1985;150:76-85.
83. Hollander A, Doekes G, Heederik D. Cat and dog allergy and total IgE as risk factors of laboratory animal allergy. J Allergy Clin Immunol 1996;98:545-554.
84. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680-685.
85. Boleij J, Buringh E, Heederik D, Kromhout H. Occupational hygiene of chemical and biological agents. Amsterdam: Elsevier, 1995:-285.
86. Hollander A, Heederik D, Doekes G. Respiratory allergy to rats: exposure-response relationships in laboratory animal workers. Am J Respir Crit Care Med 1997;155:562-567.
87. Kromhout H, Swuste P, Boleij JSM. Emperical modelling of chemical exposure in the rubber manufactering industry. Ann Occup Hyg 1994;38:3-22.
88. Preller L, Heederik D, Kromhout H, Boleij JSM, Tielen MJM. Determinants of dust and endotoxin exposure of pig farmers: development of a control strategy using empirical modelling. Ann Occup Hyg 1995;39:545-557.
89. Eisen EA, Smith TJ, Wegman DH, Louis TA, Froines J. Estimation of long term dust exposures in the vermont granite sheds. Am Ind Hyg Assoc J 1984;45:89-94.
90. Preller L, Kromhout H, Heederik D, Tielen MJM. Modelling long-term average exposure in occupational exposure-response analysis. Scan J Work Environ Health 1995;21:504-512.
91. Smid T, Heederik D, Mensink G, Houba R, Boleij JSM. Exposure to dust, endotoxin and fungi in the animal feed industry. Am Ind Hyg Assoc J 1992;53:362-368.
92. Ohman JL, Hagberg K, MacDonald MR, Jones RR, Paigen BJ, Kacergis JB. Distribution of airborne mouse allergen in a major mouse breeding facility. J Allergy Clin Immunol 1994;94:810-817.
93. Nieuwenhuijsen MJ, Gordon S, Harris J, Tee RD, Venables KM, Newman Taylor AJ. Determinants of airborne allergen exposure in an animal house. Occup Hyg 1995;1:317-324.
94. Platts-Mills TAE, Heyman PW, Longbottom JL, Wilkins SR. Airborne allergens associated with asthma: Particle sizes carrying dust mite and rat allergens measured with a cascade impactor. J Allergy Clin Immunol 1986;77:850-857.

95. Jones RB, Kacergis JB, MacDonald MR, McKnight FT, Turner WA, Ohman JL, Paigen B. The effect of relative humidity on mouse allergen levels in an environmentally controlled mouse room. Am Ind Hyg Assoc J 1995;56:398-401.
96. Swanson MC, Campbell AR, O'Hollaren MT, Reed CE. Role of ventilation, air filtration, and allergen production rate in determining concentrations of rat allergens in the air of animal quarters. Am Rev Respir Dis 1990;141:1578-1581.
97. Kacergis JB, Jones RB, Reeb CK, Turner WA, Ohman JL, Ardman MR, Paigen B. Air quality in an animal facility: particulates, ammonia, and volatile organic compounds. Am Ind Hyg Assoc J 1996;57:634-640.
98. Corn M, Esmen NA. Workplace exposure zones for classification of employees exposures to physical and chemical agents. Am Ind Hyg Assoc J 1979;40:47-57.
99. Mark D, Vincent JH. A new personal sampler for airborne total dust in workplaces. Ann Occup Hyg 1986;30:89-102.
100. Hollander A, van Run P, Spithoven J, Doekes G, Heederik D. Exposure of laboratory animal workers to airborne rat and mouse urinary allergens. Clin Exp Allergy (in press).
101. Nieuwenhuijsen MJ, Gordon S, Harris JM, Tee RD, Venables KM, Newman Taylor AJ. Variation in rat urinary aeroallergen levels explained by differences in site, task and exposure group. Ann Occup Hyg 1995;39:819-825.
102. Vincent JH, Mark D, Miller B, Armbuster L, Ogden TL. Aerosol inhalability at higher wind speeds. J Aerosol Sci 1990;21:577-586.
103. Sakaguchi M, Inouye S, Miyazawa H, Kamimura H, Kimura M, Yamazaki S. Particle size of airborne mouse crude and defined allergens. Lab animal science 1989;39:234-236.
104. Lippmann M, Yeates DB, Albert RE. Deposition, retention, and clearance of inhaled particles. Br J Ind Med 1980;37:337-362.
105. Renström A, Karlsson AS, Larsson PH, Malmberg P. High prevalence of symptoms and specific IgE to laboratory animal allergens in spite of low exposure among research personnel. Eur Respir J 1996;9, suppl 23:120s
106. Helsel DR. Less than obvious, statistical treatment of data below the detection limit. Environ Sci Technol 1990;24:1767-1774.
107. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;i:307-310.
108. Vaughan NP, Chalmers CP, Botham RA. Field comparison of personal samplers for inhalable dust. Ann Occup Hyg 1990;34:553-573.
109. Kenny LC, Chalmers C, Gonzalez-Fernandez E, Kromhout H, Lidèn G, Mark D, Riediger G, Prodi V. A collaborative European study of personal inhalable aerosol samples preformance. Ann Occup Hyg (in press).
110. Gordon S, Tee RD, Newman Taylor AJ. Analysis of the allergenic composition of rat dust. Clin Exp Allergy 1996;26:533-541.
111. Dreborg S. Skin tests used in type I allergy testing. Position paper. Allergy 1989;44, suppl 10:1-59.
112. El Shami AS, Alaba O. Liquid-phase in vitro allergen-specific IgE assay with situ immobilization. Advances in the Biosciences 1989;74:191-201.
113. Doekes G, Douwes J, Wouters I, de Wind S, Houba R, Hollander A. Enzyme immunoassays for total and allergen specific IgE in population studies. Occup Environ Med 1996;53:63-70.
114. Lee J, Chia KS. Estimation of prevalence rate ratios for cross sectional data: an example in occupational epidemiology. Br J Ind Med 1993;50:861-862.

115. Axelson O, Fredriksson M, Ekberg K. Use of the prevalence ratio versus the prevalence odd ratio as a measure of risk in cross sectional studies. Occup Environ Med 1994;51:574
116. Hoolboom H, Vermeer LPH. Allergie door het omgaan met proefdieren. Leiden: NIPG-TNO, 1985:
117. Zetterström O, Osterman K, Machado L, Johansson SGO. Another smoking hazard: raised serum IgE concentration and increased risk of occupational allergy. Br Med J 1981;283:1215-1217.
118. Venables KM, Topping MD, Howe W, Luczynska CA, Hawkins R, Newman Taylor AJ. Interaction of smoking and atopy in producing specific IgE antibody against a hapten protein conjugate. Br Med J 1985;290:201-204.
119. Gerrard JW, Heiner DC, Ko CG, Mink J, Meyers A, Dosman JA. Immunoglobulin levels in smokers and non-smokers. Ann Allergy 1980;44:261-262.
120. Chan-Yeung M, Vedal S, Lam S, Enarson D. Immediate skin reactivity and its relationship to age, sex, smoking, and occupational exposure. Arch Environ Health 1985;40:53-57.
121. Juniper CP, How MJ, Goodwin BFJ, Kinshott AK. Bacillus subtilis enzymes: a 7-year clinical epidemiological and immunological study of an industrial allergen. J Soc Occup Med 1977;27:3-12.
122. Chan-Yeung M, Lam S, Keorner S. Clinical features and natural history of occupational asthma due to Western red cedar (Thuja plicata). Am Rev Respir Dis 1982;72:411-415.
123. Burge PS. Diagnosis of occupational asthma. Clin Exp Allergy 1989;19:649-652.
124. Burge PS. Use of serial measurements of peak flow in the diagnosis of occupational asthma. In: Eisen AE, ed. Occupational medicine: spirometry (Vol. 8). Philadelphia: Hanley & Belfus, Inc. 1993:279-294.
125. Moscato G, Godnic-Cvar J, Maestrelli P. Statement on self-monitoring of peak expiratory flows in the investigation of occupational asthma. Eur Respir J 1995;8:1605-1610.
126. Cartier A. Definition and diagnosis of occupational asthma. Eur Respir J 1994;7:153-160.
127. Venables KM, Burge PS, Davison AG, Newman Taylor AJ. Peak flow rate records in surveys: reproducibility of observers' reports. Thorax 1984;39:828-832.
128. Higgins BG, Britton JR, Chinn S, Jones TD, Jenkinson D, Burney PGJ, Tattersfield E. The distribution of peak expiratory flow variability in a population sample. Am Rev Respir Dis 1989;140:1368-1372.
129. Revsbech P, Andersen G. Diurnal variation in peak expiratory flow rate among grain elevator workers. Br J Ind Med 1989;46:566-569.
130. Lee HS, Ng TP, NG YL, Phoon WH. Diurnal variation in peak expiratory flow rate among polyvinylchloride compounding workers. Br J Ind Med 1991;48:275-278.
131. Lee HS, Phoon WH. Diurnal variation in peak expiratory flow rate among workers exposed to toluene diisocyanate in the polyurethane foam manufacturing industry. Br J Ind Med 1992;49:423-427.
132. Milton DK, Wypÿ D, Kriebel D, Walters MD, Hammond SK, Evans JS. Endotoxin exposure-repsonse in a fiberglass manufacturing facility. Am J Ind Med 1996;29:3-13.
133. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report working party standardization of lung function tests European Community for Steel and Coal. Official statement of the European Respiratory Society. Eur Respir J 1993;6 suppl 16:5-40.

134. Malo JL, Coté J, Cartier A, Boulet LP, L'Archevêque J, Chan-Yeung M. How many times per day should peak expiratory flow rates be assessed when investigating occupational asthma? Thorax 1993;48:1211-1217.
135. Hetzel MR, Clark TJH. Comparison of normal and asthmatic circadian rhythms in peak expiratory flow rate. Thorax 1980;35:732-738.
136. Boezen HM, Schouten JP, Postma DS, Rijcken B. Relation between respiratory symptoms, pulmonary function and peak flow variability in adults. Thorax 1995;50:121-126.
137. Quackenboss JJ, Lebowitz MD, Kryzanowski M. The normal range of diurnal changes in peak flow expiratory flow rates - Relationship to symptoms and respiratory disease. Am Rev Respir Dis 1991;143:323-330.
138. Reddel HK, Salome CM, Peat JK, Woolcock AJ. Which index of peak expiratory flow is most useful in the management of stable asthma? Am J Respir Crit Care Med 1995;151:1320-1325.
139. Boezen HM, Postma DS, Schouten JP, Kerstjens AM, Rijcken B. PEF variability, bronchial responsiveness and their relation to allergy markers in a random population (20 - 70 yr). Am J Respir Crit Care Med 1996;154:30-35.
140. Higgins BG, Britton JR, Chinn S, Cooper S, Burney PGJ, Tattersfield E. Comparison of bronchial reactivity and peak expiratory flow variability measurements for epidemiologic studies. Am Rev Respir Dis 1992;145:588-593.
141. Neukirch F, Liard R, Segala C, Korobaeff M, Henry C, Cooreman J. Peak expiratory flow variability and bronchial responsiveness to methacholine. An epidemiologic study in 117 workers. Am Rev Respir Dis 1992;146:71-75.
142. Hauser R, Daskalakis C, Christiani DC. A regression approach to the analysis of serial peak flow among fuel oil ash exposed workers. Am J Respir Crit Care Med 1996;154:974-980.
143. Malo JL, Trudeau C, Ghezzo H, L'Archevêque J, Cartier A. Do subjects investigated for occupational asthma through serial peak expiratory flow measurements falsify their results? J Allergy Clin Immunol 1995;96:601-607.
144. Hyland ME, Kenyon CAP, Allen R, Howarth P. Diary keeping in asthma: comparison of written and electronic methods. BMJ 1993;306:487-489.
145. Quirce S, Contreras G, Dybuncio A, Chan-Yeung M. Peak expiratory flow monitoring is not a reliable method for establishing the diagnosis of occupational asthma. Am J Respir Crit Care Med 1995;152:1100-1102.
146. Zureik M, Liard R, Ségala C, Henry C, Korobaeff M, Neukirch F. Peak expiratory flow rate variability in population surveys. Does the number of assessments matter? Chest 1995;107:418-423.
147. Venables KM, Davison AG, Browne K, Newman Taylor AJ. Pseudo-occupational asthma. Thorax 1989;44:760-761.
148. Wegman DH. Issues in the epidemiologic evaluation of exposure-response relationships. In: Rappaport SM, Smith TJ, eds. Exposure assessment for epidemiology and hazard control. Chelsea, MI, US: Lewis, 1991:159-174.
149. Rothman KJ. Modern epidemiology. Boston/Toronto: Little, Brown & Company, 1986.
150. Nordman H. Atopy and work. Scand J Work Environ Health 1984;10:481-485.
151. Terr AI. The atopic worker. Clin Rev Allergy 1986;4:267-288.
152. Newill CA, Evans III R, Khoury MJ. Preemployment screening for allergy to laboratory animals: epidemiologic evaluation of its potential usefulness. J Occup Med 1986;28:1158-1164.

153. Nordman H. Atopy and preemployment screening. Eur J Respir Dis 1987;71 suppl 154:102-110.
154. Seixas NS, Robins TG, Moulton LH. The use of geometric and arithmetic mean exposures in occupational epidemiology. Am J Ind Med 1988;14:465-477.
155. Hunskaar S, Fosse RT. Allergy to laboratory mice and rats: a review of the pathophysiology, epidemiology and clinical aspects. Lab Animals 1990;24:358-374.
156. Veen MJv, Mulder M, Witteman AM, Ree Rv, Aalberse RC, Jansen HM, Zee JSv. False-positive skin prick test responses to commercially available dog dander extracts cause by contamination with house dust mite (Dermatophagoides pteronyssinus) allergens. J Allergy Clin Immunol 1996;98:1028-1034.
157. Hunskaar S, Fosse RT. Allergy to laboratory mice and rats: a review of its prevention, management, and treatment. Lab Animals 1993;27:206-221.
158. Yamauchi C, Obara T, Fukuyama N, Ueda T. Evaluation of a one-way airflow system in an animal room based on counts of airborne dust particles and bacteria and measurement of ammonia levels. Lab Animals 1989;23:7-15.
159. Gordon S, Wallace J, Cook A, Tee RD, Newman Taylor AJ. Reduction of exposure to laboratory animal allergens in the workplace (submitted).
160. French MAH, Harrison G. Serum IgG subclass concentration in healthy adults: a study using monoclonal antisera. Clin Exp Immunol 1984;56:473-475.
161. Sakaguchi M, Inouye S, Miyazawa H, Kamimura H, Kimura M, Yamazaki S. Evaluation of dust respirators for elimination of mouse aeroallergens. Lab animal science 1989;39:63-66.
162. Newill CA, Koegel AE, Prenger VL, Evans R, Corn M. Utilization of personal protective equipment by laboratory personnel at a large medical research institution. Appl Ind Hyg 1989;4:205-209.
163. Slovak AJM, Orr RG, Teasdale EL. Efficacy of the helmet respiratory in occupational asthma due to laboratory animal allergy (LAA). Am Ind Hyg Assoc J 1985;46:411-415.
164. Lutsky V, Kalbfleisch JH, Fink JN. Occupational Allergy to laboratory animals: employer practices. J Occup Med 1983;25:372-376.
165. Kort de WLAM. Personnel selection through pre-employment medicals. Thesis University of Amsterdam, 1993.

SUMMARY

INTRODUCTION

The working environment often contains allergenic agents and population surveys have shown that exposure to these agents involves a high risk of developing occupational allergies. High molecular weight agents, which are usually proteins derived from plants, micro-organisms or animals, are a major group of occupational allergens. In general, the mechanism of an allergic reaction to high molecular weight agents is IgE-dependent. Clinical manifestations of an IgE-mediated occupational allergy are asthma, rhinitis, conjunctivitis, and urticaria. Of all agents, urinary proteins from laboratory animals have been suggested to be the most common high molecular weight allergens causing occupational asthma. In addition, epidemiological studies among laboratory animal workers reported high prevalence rates these work related allergic symptoms, also described as Laboratory Animal Allergy (LAA), ranging from 11 to 44 percent. For some of the affected workers, the symptoms were so severe that direct or even indirect contact with laboratory animals became impossible.

The many recently performed studies on LAA leave no question that persons working with laboratory animals are at risk of developing occupational allergy. The question of concern is what the exact magnitude of this risk is and what factors determine the risk. It seems obvious that exposure level and/or duration of exposure determine the risk of developing LAA to a large extent. The objective of this thesis is to study exposure-response relationships of LAA and their modification by host factors, such as atopy, gender and smoking. The majority of the cases of allergic disease among laboratory animal workers is caused by rat and mouse allergens, probably because these animals are most commonly used in experimental studies. Therefore, this thesis is focused on allergy due to working with rats or mice. Several research questions were derived from the objective of this study,

1. To what level of airborne Rat (RUA) and Mouse Urinary Allergens (MUA) are Dutch laboratory animal workers exposed?
2. Which factors, e.g. job title or task performed, are affecting the RUA and MUA exposure levels?
3. What is the prevalence rate of LAA in the Netherlands?
4. Is the level of animal allergen exposure related to the prevalence rate of LAA?
5. Which host factors are associated with the prevalence rate of LAA and to what extent do they influence the slope of the exposure-response relationship?

STUDY DESIGN

In order to answer these research questions, a cross-sectional study among laboratory animal workers was performed. The study consisted of five parts. (1) questionnaire, questions were asked about personal history of allergic symptoms to common allergens, history of allergic symptoms to laboratory animals, smoking history and intensity of contact with laboratory and domestic animals. (2) skin prick testing (SPT) was performed using five common allergens (house dust mites, grass pollen, tree pollen, cat fur and dog fur) and four occupational allergens (rat urine, rat fur, mouse urine, mouse fur). (3) measurement of total IgE and specific IgE to rat and mouse urinary allergens were performed. (4) lung function was measured by spirometry. In addition, the workers recorded their peak expiratory flow (PEF) several times a day, during a period of fourteen days. (5) exposure measurements were performed in all participating facilities. The exposure measurements consisted of ambient air measurements, i.e. stationary particle size and total dust sampling, and personal inhalable dust sampling during shifts and during specific tasks.

Employees from laboratory animal facilities of four universities, two non-university research institutes, one pharmaceutical company, and students of a laboratory school participated in the study. All subjects working with small laboratory animals or having contact with material from these animals were invited to participate. Of approximately 750 eligible subjects 579 (77%) participated. Questionnaires were completed by 577 subjects (99.6%), 577 (99.6%) gave a blood sample and 542 (94%) were skin prick tested. A completed questionnaire as well as SPT results were available for 540 participants. Of these 540 participants, 458 (85%) worked with rats or had done so in the past and 377 (70%) worked with mice or had done so in the past. Three hundred and forty two individuals (63%) worked or used to work with mice as well as rats. The group of 540 laboratory animal workers was used in the papers presented in this thesis.

EXPOSURE TO RAT AND MOUSE AEROALLERGENS

Exposure measurements were performed in seven participating facilities. In order to estimate the allergen exposure levels, two 'sandwich' enzyme immunoassays have been developed to measure RUA and MUA. In the immunoassays rabbit antibodies against rat urinary proteins or mouse urinary proteins were used to detect the allergens in dust samples. The rabbit antibodies were analysed by SDS-PAGE and immunoblotting and compared with IgE antibodies from sensitised laboratory animal workers. The rabbit antibodies were highly specific for both rat or mouse urinary proteins and reacted with all IgE-binding allergens in the urinary protein preparation. As a result, both methods were very specific and cross-reactivity with proteins which

could be simultaneously found in rat and mouse rooms will be of minor importance in both assays. In addition, both assays were very sensitive, with a detection limits of 0.075 ng urinary protein per ml of extraction buffer (Chapter 2).

The allergen concentrations presented in this thesis can not be compared with reported levels in other studies. Large differences can be found between the various methods to measure rat and mouse aeroallergens (Chapter 4). The reported allergen concentrations presented by the various research institutes depended largely on the extraction method, reference preparation, type of antibodies and type of immunoassay used.

Large differences in ambient air RUA and MUA concentrations were found when various animal rooms were compared (Chapter 3). This variability in allergen concentration could be explained for more than half by the number of animals in the room. The RUA and MUA level increased approximately 1.6 and 1.8 times when the number of rats or mice was doubled, respectively. Additionally, the activity of the animals has a large effect on the ambient air allergen level in rooms. The inverse day/night rhythm, which makes rats more active during working hours, resulted in 11 times higher RUA levels in the rooms. Chapter 3 also showed that in rat and mouse rooms where filter tops were used, the RUA and MUA concentrations were 6 and 17 times lower compared to similar rooms without filter top cages.

Personal dust samples were taken during shifts and during the performance of various tasks. Animal caretakers appeared to experience the highest RUA and MUA exposure (Chapter 2). However, the RUA and MUA levels varied strongly from day to day and also between workers with similar jobs. The large variability in exposure within each job was striking and indicates that information regarding the job performed is probably not sufficient to be used as a measure of exposure in epidemiological studies. The variability appeared to be partly due to the wide range of tasks performed.

On Mondays the ambient air RUA and MUA levels were significantly elevated compared to the levels on the other days of the week (Chapter 3). In addition, the personal RUA and MUA exposure levels were also elevated on Mondays. On Mondays, approximately 70% of the time is spent on cleaning out dirty cages and changing animals into new cages. These tasks, which could be summarised as working with contaminated bedding material or handling large numbers of conscious animals, were associated with the highest aeroallergen exposure (Chapter 3). Therefore, the proportion of time spent on these tasks will determine the RUA or MUA exposure of the workers to a large extent. However, considerable variability in allergen concentration within each task remained unexplained. Some of this variability may be due to differences between sites. Possible factors are the number of animal present in the facility or the presence of special control measures, like isolators or filter top cages.

EPIDEMIOLOGICAL STUDY

In our study 18.8% of the rat workers reported allergic symptoms caused by rats (chest tightness (asthma), eye/nose or skin symptoms, Chapter 5). Among mouse workers allergic symptoms caused by mice were reported by 10.1% of the workers. When allergic symptoms caused by other small animals, like rabbits and guinea pigs, are included, 23% of the all workers reported work related allergic symptoms. Nose/eye symptoms were reported most frequently (rats 16.8%, mice 9.0%), followed by skin symptoms (rats 10.7%, mice 4.2%) and asthmatic symptoms (rats 6.1%, mice 3.2%).

The allergic symptoms due to rat allergens were mostly present immediately after contact with laboratory animals. In this immediate-type or 'atopic' reaction IgE antibodies play an important role. The presence of IgE, i.e. sensitisation, was measured by skin prick tests and immunochemical analysis of serum samples. Sensitisation to rat allergens was present in 18.2% of the rat workers and sensitisation to mouse allergens was present in 10.7% of the mouse workers. Symptoms, especially asthmatic symptoms, and sensitisation correlated strongly. Of the rat workers with allergic symptoms, 69% were sensitised to rat allergens. For workers with asthmatic symptoms up to 82% were sensitised. For mouse workers these associations were less pronounced, 39% and 58%, respectively (Chapter 5).

An important host or person's related factor is the predisposition to develop an atopic reaction, i.e. to produce IgE antibodies. Various indices of 'atopy' were used in our study (Chapter 5). All indices were associated with rat and mouse allergy. Rat and mouse allergy were defined as having allergic symptoms due to working with rats or mice and being sensitised to rat or mouse allergens. We found that allergy to cats and dogs, defined by SPT or questionnaire, was highly associated with rat and mouse allergy. In contrast, the prevalence rates of rat and mouse allergy in workers without SPT responses to cat and dog fur but positive to at least one of the other common allergens, i.e. grass pollen, tree pollen, or house dust mite, were comparable with those among workers with no SPT response to any common allergens.

Elevated total IgE (≥ 100 kU/l) was also strongly associated with the prevalence rate of rat and mouse allergy. When three indices of atopy, i.e. 'history of allergic symptoms to pets', 'positive SPT response to cat or dog fur' and 'elevated total IgE', were combined, a very strong association with the prevalence rate of rat and mouse allergy was found (Chapter 5). The prevalence rates of rat and mouse allergy were 1.8% and 0.43%, respectively, when none of the three 'atopy associated risk factors' was present. The prevalence rates of rat and mouse allergy increased with increasing number of risk factors present, from respectively 21% and 6.3% with one 'atopy associated risk factor', to respectively 46% and 17% with at least two 'atopy

associated risk factors'.

Chapter 6 clearly showed that a relationship between level of exposure to rat urinary aeroallergens and prevalence rate of sensitisation to rat allergens exists. The prevalence rates of sensitisation to rat allergens for the atopic workers were about 15, 9.5 and 7.3 times higher in the high, medium and low exposure group, respectively, compared to an internal reference group, i.e. animal workers who never worked with rats, but worked with other animals. The association between allergen exposure and sensitisation to rat allergens (Chapter 6) clearly varied between workers with and without 'atopy associated risk factors'. In addition, the slope of the exposure-response relationship was steeper in workers with atopy associated risk factors, which suggested a combined effect of exposure and the atopy associated risk factor on the development of LAA. The atopy associated risk factor should therefore be considered as effect modifier of exposure.

Other important host factors were gender and smoking. After taking into account exposure and atopy, sensitisation to rat allergens was about twice as prevalent in men and about 1.6 times more prevalent among smokers (Chapter 6). Both differences were, however, not statistically significant.

On each day during a period of two weeks the participants had to record their peak expiratory flow (PEF) and had to report working with laboratory animals (yes/no) (Chapter 7). This chapter showed that the PEF of workers who reported asthmatic symptoms caused by rats decreased significantly on days working with laboratory animals. This effect was more pronounced among workers with a late response, i.e. the presence of asthmatic symptoms several hours after working with rats. In addition, workers with asthmatic symptoms were also more likely to have a higher PEF variability than workers without asthmatic symptoms, even if these workers without asthmatic symptoms experienced skin, eye or nose symptoms due to working with rats. No difference in PEF variability between days with and without animal contact was observed.

CONCLUSIONS

Laboratory animal allergy is a major health problem of laboratory animals workers. Cat and dog allergy, and elevated total IgE, and level of exposure are important risk factors of rat allergy. In the cross-sectional analysis all parameter were measured at the same time, making it impossible to verify that the factors mentioned above precede the development of rat allergy and are indeed risk factors. This has to be confirmed in analyses of follow-up data.

SAMENVATTING

INLEIDING

Stoffen die allergieën veroorzaken, allergenen, hebben vaak een natuurlijke oorsprong. Dat wil zeggen dat ze afkomstig zijn van planten, dieren of micro-organismen. Dergelijke stoffen kunnen ook op de werkplek voorkomen. Door het contact met allergenen afkomstig van proefdieren kunnen werknemers, zoals dierverzorgers, biotechnici of onderzoekers, een allergie ontwikkelen; proefdierallergie. Uit eerder onderzoek uitgevoerd in het buitenland blijkt dat één op de vijf proefdierwerkers allergische klachten heeft tijdens of na het werken met proefdieren. Deze klachten kunnen verschillend van aard zijn en worden dan ook ingedeeld in drie groepen; (1) klachten van de neus en ogen: een prikkelende of verstopte neus, niesbuien, en prikkende (jeukende) of rode ogen, (2) huidklachten: een jeukende of rode huid en (3) astmatische klachten: kortademigheid, benauwdheid en piepen op de borst.

Een belangrijke vraag met betrekking tot het ontstaan van proefdierallergie is of bij een hogere blootstelling aan allergenen afkomstig van proefdieren, het risico om een proefdierallergie te ontwikkelen groter wordt. Dit is belangrijk voor de preventie van proefdierallergie. Blootstelling verlagende maatregelen kunnen er dan voor zorgen dat het risico om een allergie te ontwikkelen wordt verlaagd. Het centrale doel van het onderzoek beschreven in dit proefschrift is dan ook de relatie tussen blootstelling en risico van proefdierallergie in kaart te brengen. De uiteindelijke onderzoeksvragen waren:

1. Hoe hoog is de blootstelling aan rat en muisallergenen in Nederlandse proefdiercentra?
2. Wat is de invloed van factoren, die met de aard van het werk te maken hebben, op de blootstelling aan deze allergenen? Onder deze factoren verstaan we onder andere iemands specifieke werkzaamheden, de ruimte waarin iemand werkt, de aanwezigheid van afzuiging of ventilatie en het aantal en soort proefdieren, waarmee iemand werkt.
3. Hoeveel mensen hebben last van allergische klachten, veroorzaakt door het werken met proefdieren? Met andere woorden: Hoe groot is het probleem van proefdierallergie in Nederland?
4. Is de mate van blootstelling aan proefdierallergenen gerelateerd aan het voorkomen van proefdierallergie?

5. Wordt een eventuele blootstelling-respons relatie beïnvloed door persoonlijke eigenschappen van de werknemers, zoals rookgewoonte, geslacht of atopie. Onder atopie wordt de aanleg om een allergie te ontwikkelen verstaan.

OPZET VAN HET ONDERZOEK

Om deze onderzoeksvragen te kunnen beantwoorden is een dwarsdoorsnede-onderzoek uitgevoerd onder proefdierwerkers. Het gezondheidskundig onderzoek bestond uit een viertal elementen. (1) vragenlijst; hierin stonden vragen over allergische klachten, rookgewoonte, het hebben van huisdieren, en werkhistorie. (2) huidpriktest; door enkele kleine prikjes in de onderarm is de allergische reactie bekeken op 'algemene' allergenen (boompollen, graspollen, huisstofmijt, kattehaar en hondehaar) en werkgebonden allergenen (ratte-urine, rattehaar, muize-urine en muizehaar). (3) bloedonderzoek; IgE-antilichamen een rol spelen bij een allergische reactie en daarom is in het serum de aanwezigheid van IgE-antilichamen tegen allergenen afkomstig uit ratte- en muize-urine gemeten. Daarnaast is ook het totale IgE-antilichaamgehalte gemeten. (4) piekstroom; de maximale snelheid waarmee kan worden uitgeblazen is gedurende 14 dagen regelmatig gemeten. Alle deelnemers kregen een piekstroommeter mee en moesten hierin enkele malen per dag blazen en de resultaten daarvan noteren in een dagboekje.

Daarnaast zijn er ook blootstellingsmetingen uitgevoerd. Hiervoor zijn er grote pompen neergezet in verschillende ruimtes in de proefdiercentra. Deze zuigen de lucht aan en vervolgens zijn de allergenen op filters bepaald. Ook heeft een deel van de proefdierwerkers een pompje gedragen, om te kijken hoeveel stof en allergenen de proefdierwerkers inademen.

Proefdierwerkers afkomstig van vier universiteiten, twee onderzoeksinstituten, een farmaceutisch bedrijf en studenten van een hogeschool hebben deelgenomen aan het onderzoek. Alle proefdierwerkers die werken met kleine proefdieren (ongeveer 750) waren uitgenodigd om deel te nemen aan het onderzoek. Hiervan hebben uiteindelijk 579 (77%) deelgenomen aan het onderzoek. Van uiteindelijk 540 werknemers waren zowel vragenlijst- als huidpriktestgegevens beschikbaar. De gegevens van deze groep werknemers is uiteindelijk gebruikt in het onderzoek beschreven in dit proefschrift.

BLOOTSTELLING

De IgE-antilichamen die verantwoordelijk zijn voor de allergische reactie bij werknemers, zijn voornamelijk gericht tegen eiwitten in de urine van de proefdieren. In hoofdstuk 2 worden twee immunochemische methoden beschreven om rat- and muisallergenen te meten in luchtmonsters. Beide methoden, die gebruik maken van

antilichamen afkomstig uit tegen rat- and muisallergenen geïmmuniseerde konijnen, bleken zeer specifiek te zijn. De gebruikte konijne-antilichamen gaven een positieve reactie op alle allergenen waarop ook allergische werknemers positief reageerden. We kunnen daarom met grote zekerheid zeggen dat inderdaad rat- of muisallergenen zijn gemeten. Bovendien zijn beide methoden zeer gevoelig en allergeenconcentraties tot 0,070 ng eiwit per ml extractiebuffer zijn nog meetbaar.

In hoofdstuk 4 hebben we onze methoden om rat- en muisallergenen te meten vergeleken met methoden van een Engels en een Zweeds onderzoeksinstituut. De correlatie tussen de methoden van de drie instituten was goed, hoewel er grote verschillen tussen de gevonden niveaus bleken te zijn. Vooral de methode om ratallergenen te meten, ontwikkeld door het Engelse instituut, gaf zeer hoge concentraties, meer dan 1000 keer hogere concentraties dan onze methode en de methode van het Zweedse instituut. Deze gevonden verschillen zijn toe te schrijven aan de manier waarop de filters werden geëxtraheerd, de immunochemische techniek die werd toegepast, bijvoorbeeld een 'inhibitie' of een 'sandwich immunoassay', en de antilichamen die werden gebruikt, zoals bijvoorbeeld de IgE-antilichamen van gesensibiliseerde werknemers of antilichamen afkomstig van geïmmuniseerde konijnen. Uit hoofdstuk 4 blijkt dus dat de allergeenconcentraties uit verschillende studies moeilijk onderling vergelijkbaar zijn.

Om eventueel een reductie van de blootstelling tot stand te brengen, is het belangrijk om te weten wanneer en waarom er veel allergenen in de lucht aanwezig zijn. Daarom werd in 40 proefdierkamers verspreidt over 7 deelnemende instituten de hoeveelheid allergenen gemeten (hoofdstuk 3). Analyse van de resultaten liet zien dat het aantal dieren in een kamer een belangrijke invloed heeft op de gemeten concentraties. Een verdubbeling van het aantal dieren zorgt ervoor dat de allergeenconcentratie ongeveer 1.7 keer toeneemt. Dit is voor het aantal muizen en ratten ongeveer gelijk. Een andere belangrijke factor voor de verschillen in concentratie is de activiteit van de dieren. In een rattenkamer met een omgekeerd dag/nacht ritme (infrarood licht) was de allergeenconcentratie ongeveer 11 keer hoger dan in een vergelijkbare kamer zonder infrarood licht. Dit komt waarschijnlijk omdat de ratten 's nachts veel actiever zijn en meer stof doen opdwarrelen. Daarnaast was de allergeenconcentratie op maandag ongeveer twee keer zo hoog als de concentratie op andere dagen van de week. Op maandag worden de dieren overgezet in schone bakken en dit bleek een handeling te zijn waarbij een hoge blootstelling werd gemeten. Het is ook mogelijk dat bepaalde maatregelen zijn genomen in een ruimte, waardoor de concentratie in die ruimte verlaagd is. Zo bleek in ruimtes waarin de kooi voorzien was van een filterkap een ongeveer 6 keer lagere ratallergeenconcentratie en 17 keer lagere

muisallergeenconcentratie voor te komen. Met de bovenstaande variabelen kunnen we het merendeel van de verschillen in concentratie tussen de verschillende proefdierkamers verklaren. De mate van ventilatie in de dierkamer, de grote van de kamer en de luchtvochtigheid hadden geen invloed op de gevonden concentraties.
Het is belangrijk te weten wat de allergeenblootstelling is van proefdierwerkers. Daarom heeft een groot aantal proefdierwerkers tijdens hun werk een pompje gedragen. Uit deze persoonlijke metingen bleek dat het belangrijk is welke werkzaamheden iemand uitvoert (hoofdstuk 3). Zo zijn de allergeenconcentraties tijdens het overzetten van ratten en muizen vele malen hoger dan de concentraties die normaal in de ruimtes worden gemeten. Omdat het overzetten van dieren vaak op maandag wordt gedaan, verklaart dit ook waarom op maandagen hogere concentraties werden gevonden in de proefdierkamers. Omdat de allergenen hoofdzakelijk aanwezig zijn in de urine van dieren, werden ook hoge allergeenconcentraties gevonden tijdens het uitmesten ofwel schoonmaken van de kooien. De conclusie is dat tijdens het hanteren van grote aantallen levende dieren en/of smerig beddingmateriaal, de hoogste allergeenconcentraties aanwezig zijn. Daarnaast is het zeer belangrijk in welk proefdiercentrum en op welke afdeling wordt gewerkt. De blootstellingsmetingen laten zien dat er grote verschillen zijn in allergeenconcentratie tussen de verschillende proefdiercentra (hoofdstukken 2 en 3). Mogelijke verklaringen hiervoor zijn de verschillen in aantal aanwezige dieren, ventilatiesysteem, en het gebruik van filterkappen.

PROEFDIERALLERGIE

Van de 540 werknemers werkte het merendeel op het moment van het onderzoek met ratten of had ooit met ratten gewerkt, 458 (85%). Voor muizen is dit aantal een stuk kleiner, namelijk 377 (70%). Daarnaast werkten 342 mensen (63%) zowel met muizen als met ratten. Van de 458 mensen die met ratten werkte, rapporteerde 18,8% allergische symptomen. Hierbij ging het om zowel oog/neus klachten, huidklachten als om benauwdheidsklachten (astma). Voor mensen die met muizen werken was dit 10,1%. De meest voorkomende klachten waren oog/neus klachten (ratten 16,8%; muizen 9,0%), gevolgd door huidklachten (ratten 10,7%; muizen 4,2%) en de astmatische klachten (ratten 6,1%; muizen 3,2%). De astmatische klachten kwamen bijna altijd in combinatie met één of meer andere klachten voor.
Wanneer een werknemer een positieve huidpriktestreactie had en/of er IgE-antilichamen waren aangetoond, dan was deze proefdierwerker gesensibiliseerd voor het betreffende allergeen. Niet alle mensen met klachten bleken ook gesensibiliseerd. Van de deelnemers met klachten veroorzaakt door ratten was 69% ook gesensibiliseerd voor

ratallergenen (hoofdstuk 5). Dit was 82% wanneer astmatische klachten aanwezig waren. Voor klachten veroorzaakt door muizen waren de percentages beduidend lager, respectievelijk 39% en 58%. Omdat we er zeker van willen zijn dat de gerapporteerde klachten inderdaad van allergische aard zijn, beschouwen we een proefdierwerker pas allergisch voor ratten of muizen wanneer er allergische klachten zijn en de werknemer ook gesensibiliseerd is tegen rat- of muisallergenen.

Sommige mensen zijn gevoeliger dan andere mensen om een allergie te ontwikkelen. Er is dus een verschil in aanleg voor het ontwikkelen van een allergie. Deze aanleg wordt ook wel 'atopie' genoemd. Een manier om deze aanleg te bepalen is te onderzoeken of mensen een allergie hebben tegen stoffen die normaal in hun omgeving voorkomen. In ons onderzoek hebben we gekeken naar een allergie voor graspollen, boompollen, huisstofmijt, kattehaar en hondehaar. Onder mensen die een allergie hadden voor katten of honden, kwam vaker een allergie voor ratten en/of muizen voor. Opvallend was dat er geen verschil was tussen de niet atopische werknemers en werknemers met een allergie voor bijvoorbeeld graspollen, boompollen of huisstofmijt maar niet voor huisdieren (hoofdstuk 5).

Een aanleg om een allergie te ontwikkelen kan ook worden getest door te kijken naar het totale aantal IgE-antilichamen dat aanwezig is in het bloed. Een verhoogd totaal IgE niveau bleek sterk samen te hangen met een rat of muisallergie. In het verdere onderzoek beschouwen we een atopische risicofactor aanwezig, wanneer een werknemer een positieve allergietest heeft voor kat of hond, in de vragenlijst heeft aangegeven allergische klachten te hebben voor katten of honden, of een verhoogd IgE-gehalte heeft.

De persoonlijke blootstellingsmetingen gaven aan dat op basis van afdeling de mensen konden worden ingedeeld in een aantal groepen. Natuurlijk werkten niet alle deelnemers van één groep eenzelfde aantal uren per week met ratten. Daarom hebben we de gemiddelde ratallergeenconcentratie in de groepen vermenigvuldigd met het gemiddelde aantal uren per week dat met levende ratten werd gewerkt. Het aantal uren was een gemiddelde van het laatste jaar voorafgaande aan het onderzoek. Hierdoor kreeg iedere deelnemer een waarde voor zijn of haar blootstelling. In de analyses hebben we alleen de groep werknemers gebruikt, die nog maar een kort aantal jaren met proefdieren werkt Omdat de meeste deelnemers AIO's of analisten waren met een tijdelijke aanstelling van ongeveer vier jaar, hebben we vier jaar als grens genomen. Vervolgens zijn de deelnemers in drie even grote groepen verdeeld met een lage, middelmatige of hoge blootstelling. Uiteindelijk was te zien dat in de laag, midden en hoog blootgestelde groep, respectievelijk 2,2 3,3 en 10,1 keer zoveel tegen ratallergenen gesensibiliseerde werknemers voorkomen dan in de controle groep (hoofdstuk 6). De controle groep bestond uit deelnemers die nooit met ratten hebben gewerkt, maar met muizen of andere

proefdieren werken. De relatie tussen blootstelling en sensibilisatie wordt echter beïnvloed door de aanwezigheid van atopische risicofactoren. Zo was het aantal gesensibiliseerde werknemers respectievelijk 7,3 9,5 en 15 keer zoveel in de laag, midden en hoog blootgestelde groep in vergelijking met de controle groep wanneer er ook atopische risicofactoren aanwezig waren.
Daarnaast bleek dat ratallergie ongeveer 2 keer zo vaak voorkomt onder mannen en onder rokers. Echter deze effecten waren niet statistisch significant en zijn klein ten opzichte van het effect van blootstelling en atopie op het voorkomen van ratallergie. Door het geringe aantal mensen dat met muizen werkt en het geringe aantal mensen dat gesensibiliseerd was voor muisallergenen, was het niet mogelijk om de analyses ook uit te voeren voor blootstelling aan muisallergenen.
Ongeveer eenderde van de werknemers met allergische klachten door het werken met ratten had last van benauwdheidsklachten (astma). Het andere deel heeft alleen last van tranende of rode ogen of van een loopneus of niesbuien. Een astmatische reactie kan worden gemeten door regelmatig de piekstroom van een werknemer te meten (hoofdstuk 7). Alle deelnemers hebben een piekstroommeter meegekregen en 73% heeft minimaal drie keer per dag hun longfunctie gemeten gedurende een periode van minimaal negen dagen. Uit deze metingen bleek dat op dagen dat met proefdieren werd gewerkt de mensen met astmatische klachten een duidelijk lagere piekstroom hadden dan op dagen dat niet met proefdieren werd gewerkt. Dit effect was niet te zien bij de andere werknemers, ook niet wanneer er wel neus, oog of huidklachten aanwezig waren.

CONCLUSIES

We kunnen een aantal belangrijke conclusies trekken uit het onderzoek. Zo blijkt dat de mate van blootstelling een heel belangrijke rol speelt bij de ontwikkeling van proefdierallergie. Bij een hogere blootstelling is het risico om een allergie te ontwikkelen groter. De relatie tussen blootstelling aan proefdierallergenen en proefdierallergie was veel sterker voor de gevoeligere werknemers. Dit zijn werknemers met een allergie tegen huisdieren of met een verhoogde hoeveelheid IgE-antilichamen. De metingen in de hoofdstukken 3 en 6 laten echter zien dat zelfs bij een lage blootstelling nog 27% van de gevoelige werknemers gesensibiliseerd is tegen ratallergenen. Het is dus duidelijk dat er een aanzienlijke reductie van de allergeenconcentratie moet plaatsvinden om ervoor te zorgen dat zelfs de gevoeligste groep zonder verhoogd risico kan werken met proefdieren (hoofdstuk 8).

PUBLICATIELIJST

- Preller L, Hollander A, Heederik D, Brunekreef B. Exposure to bio-aerosols in the vicinity of a yeast production plant. JAPCA 1989;39:1094-1097.
- Hollander A, Heederik D, Versloot P, Douwes J. Inhibition and enhancement in the analysis of airborne endotoxin levels in various occupational environments. Am Ind Hyg Ass J 1993;54:647-653.
- Hollander A, Heederik D, Kauffman H. Acute respiratory effects in the potato precessing industry due to bio-aerosol exposure. Occup Environ Med 1994;51:73-78.
- Hollander A, Heederik D. Pothuis J. Quantification of antigen aerosol levels in the potato starch producing industry. Ann Occup Hyg 1994;38 suppl.1:911-918.
- Douwes J, Versloot P, Hollander A, Heederik D, Doekes G. The influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. Appl Environ Microbiol 1995;61:1763-1769.
- Doekes G, Douwes J, Wouters I, de Wind S, Houba R, Hollander A. Enzyme immunoassays for total and allergen specific IgE in population studies. Occup Environ Med 1996;53:63-70.
- Zock J, Hollander A, Doekes G, Heederik D. The influence of different filter elution methods on the measurement of airborne potato antigens. Am Ind Hyg Ass J 1996;57:567-570.
- Hollander A, Doekes G, Heederik D. Cat and dog allergy and total IgE as risk factors of laboratory animal allergy. J Allergy Clin Immunol 1996;89:545-554.
- Hollander A, Heederik D, Doekes G. Respiratory allergy to rats: exposure-response relationsships in laboratory animal workers. Am J Respir Crit Care Med 1997;155:562-567.
- Hollander A, van Run P, Spithoven J, Doekes G, Heederik D. Exposure of laboratory animal workers to airborne rat and mouse urinary allergens. Clin Exp Allergy (in press).
- Hollander A, Heederik D, Brunekreef B. Work related changes in peak expiratory flow among laboratory animal workers (submitted).
- Hollander A, Heederik D, Doekes G, Kromhout H. Determinants of airborne rat and mouse urinary allergen exposure (submitted).
- Kruize H, Post W, Heederik D, Martens B, Hollander A, van der Beek E. Respiratory allergy in laboratory animal workers: a retrospective cohort study using pre-employment screening data (submitted).

- Hollander A, Heederik D, Smid T. Longitudinaal longfunctieonderzoek in een scheepsschroevenbedrijf. TSG 1988;66:253-256.
- Hollander A, Run P van, Oortgiese I, Heederik D. Ontwikkeling van een immunochemische methode voor de bepaling van ratantigenen in lucht. Sdu Uitgeverij, Den Haag 1994, S 172, 17pp.
- Douwes J, Heederik D, Hollander A, Versloot P. Endotoxinen: Gezondheidseffecten, monstername en analyse van endotoxinen. Sdu Uitgeverij, Den Haag 1994, S 172-3, 33pp.
- Hollander A, Heederik D, Denen B, Pothuis J. Onderzoek naar blootstelling aan bio-aërosolen in de aardappelverwerkende industrie. Sdu Uitgeverij, Den Haag 1994, S 172-6, 46pp.

- Kamsteeg J, Heederik D, Hollander A. Kwantificering van de beroepsmatige blootstelling aan organisch stof. Sdu Uitgeverij, Den Haag 1994, S 172-8, 58pp.
- Hollander A, Run P van, Wind S de, Spithoven J, Heederik D, Doekes G. Vierjarig-onderzoek naar proefdierallergie - resultaten van één jaar onderzoek -. Biotechniek 1995:34:7-12.
- Frankhuijzen E, Hollander A, Kromhout H. De toepasbaarheid van de miniature real-time aerosol monitor (MiniRAM) in arbeidshygiënisch onderzoek. Tijdschrift voor toegepaste Arbowetenschap 1995;8:64-69.
- Neuwsbrief Proefdierallergie, nummer 1, 2 en 3. Uitgave Vakgroep Epidemiologie en Gezondheidsleer, Wageningen.

DANKWOORD

Op deze plaats wil ik iedereen bedanken die op welke manier dan ook een bijdrage heeft geleverd aan het tot stand komen van dit proefschrift. In het bijzonder wil ik Dick Heederik, Bert Brunekreef, Per Malmberg, Gert Doekes en Hans Kromhout bedanken voor de prettige begeleiding die ik tijdens mijn onderzoek en het schrijven heb gekregen. Daarnaast gaat mijn speciale dank uit naar Joost Thissen, Jack Spithoven, Siegfried de Wind, Suzanne van Gaans, Paula van Run, Isabella van Schothorst en Monique Leblanc. Het veld- en labwerk liep door hun ondersteuning zeer soepel en ik heb alle veldwerkperiodes als een aangename afwisseling ervaren. Voor de ondersteuning tijdens het veldwerk wil ik tevens de vele (werk)studenten bedanken, met name Els Frankhuijzen, Nicole Wolters, Mariëlle van Zuylen, Stefan Pasma, Susan Peelen, Susan van Kempen, Karin Leeuwinga en Marianne Veerman.
Voor het onderzoek naar proefdierallergie is de samenwerking met de bedrijfsgezondheidsdiensten van groot belang geweest. Voor deze samenwerking wil ik graag Theo Senden, Jeanine Leyser, Fons Vernooy, Marina Capel, Nelleke Bouwma, Erik van der Beek, Jack Smit, Ron Nuit, Ron Langens, Pieter Gallee, Lydia Post en Ilse Jansen bedanken. Natuurlijk had het onderzoek niet plaats kunnen vinden zonder de medewerking van de proefdiercentra en de proefdierwerkers, nogmaals van harte bedankt voor deelname aan het onderzoek.
Verder wil ik alle (ex-)medewerkers van de vakgroep Humane Epidemiologie en Gezondheidsleer bedanken, waarbij mijn speciale dank uitgaat naar mijn altijd vrolijke kamergenoten Jeroen Douwes en Remko Houba.

CURRICULUM VITAE

De schrijver van dit proefschrift werd op 1 januari 1963 geboren in Harlingen. Na het behalen van het Atheneum-B diploma aan het Develsteincollege te Zwijndrecht, begon hij met de studie Milieuhygiëne aan de Landbouwuniversiteit te Wageningen. Deze studie werd in 1988 afgerond, met doctoraalonderzoeken in de Arbeidshygiëne, Arbeidsepidemiologie en Milieu-epidemiologie. Daarnaast heeft hij stage gelopen bij de Bedrijfsgezondheidsdienst 's Hertogenbosch en de milieu-inspectie in Wellington, Nieuw Zeeland. Na zijn studie heeft hij zijn vervangende dienstplicht vervuld bij de vakgroep Humane Epidemiologie en Gezondheidsleer van de Landbouwuniversiteit Wageningen. Daar bestonden zijn voornaamste activiteiten uit het ontwikkelen en deels geven van het onderdeel Arbeidshygiëne van de post-doctorale opleiding Veiligheidskunde (Toptech Studies, Delft) en het uitvoeren van arbeidshygiënisch onderzoek naar biologische factoren. Deze laatste activiteit heeft hij in 1990 voorgezet als toegevoegd onderzoeker bij dezelfde vakgroep. In dezelfde functie heeft hij vanaf april 1992 gewerkt aan het in dit proefschrift beschreven onderzoek. Sinds 1 juni 1997 werkt hij als medewerker Arbeidshygiëne bij de afdeling Onderzoek van het Ministerie van Sociale Zaken en Werkgelegenheid.